DEVELOPMENT OF A MOUSE MODEL SYSTEM FOR IN VIVO LUNG CARCINOGENESIS

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DEVELOPMENT OF A MOUSE MODEL SYSTEM FOR IN VIVO LUNG CARCINOGENESIS

There is a real need for the determination of those factors which alter or influence the biological effects of cigarette smoking. A model system analogous to the human situation must be devised. We feel that the best model system presently available involves the use of inbred strains of mice, for the genetics, biochemistry, and types of tumor responses of this model system closely approximate the condition in humans. This system is also economically feasible. To this end we now present a series of experiments which should crystallize our concepts of this model system and thus lay the groundwork for a large scale inhalation program designed to finally establish many of the risks involved in cigarette smoking.

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I. SUMMARY PROGRESS REPORT

The experiments reported on under this contract are those described in the proposal for the contract year. The volume of work however does not reflect only those expenditures under this contract but rather also includes effort and expenditures under the larger contract running from July 1-June 30 each year. Of necessity we have attempted to have an integrated CTR program thus the work is treated as one large scientific effort although it is funded under several contracts.

The CTR-MA contracts have two basic functions:
First, to define a mouse carcinogenic model system which can be used for future inhalation studies.
Second, to undertake screening of known carcinogens, cigarette smoke condensates and condensate fractions by subcutaneous and pulmonary inoculations of mice.

A. Model System

1. Chemical Carcinogensis

a. MCA - SC Route

In order to define the mouse model systems, we have undertaken subcutaneous carcinogenesis studies with MCA to determine the relative sensitivity of several mouse strains as quickly as possible, and to compare subcutaneous and intratracheal susceptibility. This MCA-SC study (CTR-4) has been completed. Based on the MCA dose required to produce fifty percent tumors (TD_{50}) in eight months, the most susceptible strain was the C3H/f, followed by the B6C3F1 hybrid using the C3H/fMai and the C57BL/6 Cum mice. This hybrid is not available commercially but should be considered as a possible susceptible strain after it is characterized for other carcinogens. The time required for tumor development more closely paralleled that of the C3H parent. All the strains were AHH inducible. The C57BL and C57BL/6 mice were virtually negative for type C RNA gs antigen, while nearly all the C3H/f mice were gs +. The hybrid mice appear to take on the same gs antigen expression as the C3H/f parent.

b. MCA-IT Route

These same stains (C57BL, C57BL/6, C3H/f and BC3F1) have been given intratracheal inoculation of MCA (CTR-3). These mice have been on test for approximately 72 weeks. Although we lost a large number of mice during the inoculation period, we have seen very few lung tumors in mice sacrificed at varying time intervals. Within the past week we have seen several large lung tumors, however, we do not have histopathology at this time. Additional mice have been inoculated (CTR-3B, C, D) and will be observed

for tumor induction. Recent reports of the histopathology indicate that better tumor induction can be accomplished with MCA which is not sonicated so extensively, therefore, larger particles are instilled. It appears that lower doses and inoculation at two week intervals may improve survival of the mice with less pneumonia.

To study the effects of AHH inducibility on the induction of lung tumors by MCA intratracehal instillation, C57BL/6, DBA/2 and the hybrid mice have been inoculated (CTR-5). These mice have been on test up to 17 months and few tumors have been found in the few which have been sacrificed. The same problems exist with this experiment as our original CTR-3 in that the mice received MCA of too fine particle size and at too high a dose which produced early deaths. This will be repeated as CTR-39.

c. Chemical Carcinogens (Nitrosamines) (SC&IT Routes)

In defining the model system it is essential to determine the susceptibility of our selected mouse strains to nitrosamine carcinogenesis, since they are present in tobacco smoke. Three routes of administration have been studied. We inoculated neonate (less than 5 days of age) C57BL/6Cum mice intraperitoneally in an effort to induce lung and bladder tumors. After eight months, some mice treated with DMN have developed liver and lung tumors and the tumors have been submitted for histopathology. Additional mice are on test with DMN, DEN, DBN, PIP and PYR (CTR-2, 2A). Other studies have been undertaken to determine if nitrosamine can produce lung tumors when given intratracheally (CTR-18). To demonstrate whether there is any difference in susceptibility of AHH inducible mice and the non-inducible mice several strains have been injected into the lung with wax pellets containing DMN.

d. AHH Inducers

Studies were undertaken using a chemical (TCDD) which induces AHH regardless of genotype (CTR-15, 16, 17). Considerable toxicity was encountered. Both C57BL/6 and DBA/2 mice were tested to determine the effects of TCDD on subcutaneous tumor induction. The results with the TCDD are compatible with the idea that AHH induction (via TCDD) simultaneous with MCA treatment yields more tumors than MCA alone, but the results with dioxane are difficult to assess. Why a 48 hr. pretreatment with 0.010 ml dioxane should enhance MCA-induced tumorigenesis cannot be explained at this time. The fact that both the low and high TCDD levels, when given 48 hrs. before MCA, had no effect, yet dioxane was also in these treatments, indicates that whatever the effect of dioxane, it is cancelled, if TCDD is present.

We feel that a repeat of this experiment must be done. The protocol for this repeat is CTR-40.

2. Screening of Carcinogens

a. Subcutaneous Route

Earlier studies with MCA, DMBA and BP have shown the C3H/fMai strain to be the most susceptible to subcutaneous carcinogenesis. Based on these studies we undertook the screening of 14 fractions of 1R1 and 1A1 reference cigarette smoke condensates to determine their ability to induce tumors alone or with MCA as a co-carcinogen. In order to give as high doses of these fractions as possible, they were implanted as pellets using 1:1 beeswax:trioctanoin as a vehicle. This vehicle has advantages as well as disadvantages. Although it allows larger doses to be given without toxicity, the latency period for tumor development is extended. MCA (150μg) in trioctanoin induced 100% tumors in 20 weeks; while 150μg in beeswax:trioctanoin has produced only 43% tumors in 80 weeks. When 10μg MCA was given in trioctanoin, 38% tumors developed in 65 weeks; however, no tumors developed in the same period when 10μg MCA was given in beeswax:trioctanoin. When 10μg MCA in trioctanoin was injected directly into the fresh beeswax:trioctanoin pellet, as was done with the CSC fraction, 13% tumors have developed in 65 weeks. Further studies to define the relationship between vehicle, carcinogen dose and tumor latency are planned (CTR 19) using known carcinogens.

The 1R1 fractions have been on test for 82 weeks. Several tumors have developed with the fraction alone, however, with MCA (10μg) as a co-carcinogen as high as 75% tumors have been induced with the N_{CH} fraction of 1R1. The 1A1 fractions have also been tested in a similar manner. No tumors have developed with the CSC fraction alone however with 10μg MCA we have obtained up to 50% tumors. These studies with 1A1 have been on over 66 weeks. At this time, they appear to be less co-carcinogenic than the 1R1 fractions.

Fifteen whole cigarette smoke condensates were obtained through Dr. Gori at NCI and have been on test for 14 months (CTR-1B). No tumors have occurred in mice receiving only the condensates. As co-carcinogens with 10μg MCA, tumor incidences range from 0 - 67%, while 13% tumors have occurred with the 10μg MCA control.

CTR-1 Carcinogenic and Co-Carcinogenic Subcutaneous Studies with 1R1 Cigarette Smoke Condensate (CSC) Fractions in C3H/fMai Mice.

Objective:

To determine the complete and co-carcinogenic potential of 14 fractions of 1R1 cigarette smoke condensate by our most rapid subcutaneous mouse tumor assay system.

Procedure:

Various fractions of 1R1-CSC were diluted (w/v) in a 1:1 mixture of trioctanoin:beeswax and inoculated subcutaneously in C3H/fMai mice. These fractions were tested in the presence of, and without, 10 µg MCA delivered at the same site of inoculation.

Progress:

1. Mice that received subcutaneous wax-implants containing either 1R1 fractions of CSC or 10 µg of 3-methylcholanthrene (MCA) or both (co-carcinogenic) have been on test from 73 to 82 weeks. Tumor incidence and latency periods for various fractions tested are presented in Table 1. In all but two cases, tumors occurred only in mice that received both MCA and CSC fractions (co-carcinogenic). The highest tumor incidences were in fractions N_CH (75%), N_{MeOH} (53%), WA_I (50%), B_E (50%), St.Mat. (50%), and B_Ia (47%). The tumor latency periods ranged from 27.8 to 49.9 weeks for those mice that received both MCA and CSC fractions. Since only a few mice remained on test the experiment has been terminated. Histopathological studies on induced tumors are in progress.

CTR-1 Table 1: Tumor incidence and latency period for IRI fractions

Fraction[a] tested	Dose (mg)	% of CSC	w or w/o 10 μg MCA[b]	wks. on test	Tumor[c] Incidence		Avg. Latency[d] Period (wks)
St. Mat.	10.0	100.00	-	82	0/20	0	-
			+		8/16	50%	38.7
Rec. Mat.	10.0	97.16	-	82	0/20	0	-
			+		10/20	50%	28.8
$B_I b$	5.0	0.39	-	82	1/7	14%	82.0
			+		Not done	-	-
$B_I a$	5.0	0.99	-	82	1/8	5%	65.3
			+		9/19	47%	27.8
B_E	0.5	8.10	-	73	0/20	0	-
			+		10/20	50%	29.2
B_W	2.5	2.79	-	73	0/20	0	-
			+		5/14	36%	49.9
WA_I	10.0	6.52	-	82	1/20	6%	51.1
			+		6/12	50%	43.0
WA_E	10.0	7.50	-	82	0/20	0	-
			+		1/19	5%	34.8
SA_I	5.0	1.77	-	82	0/20	0	-
			+		8/20	40%	31.0
SA_E	10.0	3.30	-	82	0/20	0	-
			+		8/20	40%	28.6
SA_W	10.0	40.50	-	80	0/20	0	-
			+		6/19	32%	32.3
N_{MeOH}	10.0	4.50	-	82	0/20	0	-
			+		10/19	53%	31.8
N_{CH}	10.0	18.10	-	82	0/20	0	-
			+		12/16	75%	31.6
N_{NM}	10.0	2.70	-	82	0/20	0	-
			+		7/17	41%	36.3
150 μg MCA/BW:T		-	+	80	8/19	43%	27.4
150 μg MCA/Trioc		-	+	20	18/18	100%	10.2
10 μg MCA/BW:T		-	+	64	0/46	0%	-
10 μg MCA/Trioc		-	+	65	19/50	38%	29.0
10 μg MCA injected into BW pellet		-	+	65	6/48	13%	46.0

[a] One part of cigarette smoke fraction or chemical carcinogen was combined with a warm (78°C) mixture of Beeswax-trioctanoin (1:1) at the dilution indicated for subcutaneous inoculation.

[b] 10 μg of MCA dissolved in trioctanoin delivered at the same site that cigarette fraction was administered.

[c] Tumor incidence is the current number of tumors divided by the number of mice on test when the first tumor occurred.

[d] Latency period is the total number of weeks prior to the appearance of a tumor divided by the total number of tumors in a particular test group.

August 19, 1974

CTR-1A: Carcinogenic and Co-Carcinogenic Subcutaneous Studies with 14 Fractions of 1A1 Tobacco Smoke Condensate in C3H/fMai Mice.

Objective:

To determine the complete and co-carcinogenic potential of 14 fractions of 1A1 cigarette smoke condensate by our most rapid subcutaneous tumor assay system.

Procedure:

Various fractions of 1A1-CSC were diluted (w/v) in a 1:1 mixture of trioctanoin; beeswax and inoculated subcutaneously in C3H/fMai mice. These condensates were tested in the presence of, and without, 10 µg MCA delivered at the same site of inoculation.

Progress:

1. Mice have been on test approximately 66 weeks and tumors have occurred only in mice that received both MCA and 1A1 fractions (co-carcinogenic). Tumor incidence ranges from 5 to 50%, with fractions $B_I{}^b$ (50%) and N_{MeOH} (40%) being the highest. Latency periods ranged from 22 to 41 weeks. This experiment will be terminated at 82 weeks.

Conclusion:

The fractions of 1R1 appears to give more tumors than 1A1 when administered subcutaneously as a cocarcinogen with 10µg MCA (See Table 2).

August 12, 1974

CTR-1A Table 1: Tumor incidence and latency period for 1A1 fractions

Fraction[a] tested	Dose (mg)	% of CSC	w or w/o 10 μg MCA[b]	Wks. on test	Tumor[c] Incidence		Latency[d] Period (wks)
St. Mat.	10.0	100.00	-	67	0/18	0	
			+		3/20	15%	41
Rec. Mat.	10.0	97.16	-	66	0/16	0	
			+		4/20	20%	38
$B_I b$	5.0	.39	-	66	0/14	0	
			+		7/14	50%	23.4
$B_I a$	5.0	.99	-	66	0/17	0	
			+		5/20	25%	32.3
B_E	1.0	8.10	-	65	0/13	0	
			+		3/20	15%	35
B_W	2.5	2.79	-	67	0/15	0	
			+		2/20	10%	34
WA_I	10.0	6.52	-	67	0/18	0	
			+		5/20	20%	30
WA_E	10.0	7.50	-	67	0/21	0	
			+		4/20	20%	22.1
SA_I	5.0	1.77	-	66	0/18	0	
			+		4/20	20%	41
SA_E	10.0	3.30	-	66	0/15	0	
			+		3/20	15%	39.3
SA_W	10.0	40.50	-	68	0/12	0	
			+		1/21	5%	31
N_{MeOH}	10.0	4.50	-	66	0/20	0	
			+		8/20	40%	39
N_{CH}	10.0	18.10	-	66	0/20	0	
			+		2/20	15%	36.4
N_{NM}	10.0	2.70	-	66	0/18	0	
			+		1/20	5%	30.4
10 μg MCA/.05 ml trioc			+	65	19/50	38%	29
10 μg MCA/.05 ml trioc + BW:T			+	65	6/48	13%	46
10 μg MCA/.05 ml BW:T			+	64	0/46	0	
150 μg MCA/.05 ml trioc			+	20	13/13	100%	14
150 μg MCA/.05 ml BW:T			+	64	12/20	60%	24

a,b,c,d, See footnotes for CTR-1, Table 1.

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CTR-1, 1A: August 19, 1974

Table 2. Co-Carcinogenic Subcutaneous Pellet Studies with 1R1 and 1A1 Smoke Condensate Fractions* in C3H/fMai Mice.

CSC Stedman No. (Fraction)	CSC %	Dose** (mg)	% Tumor Incidence (65 Weeks) 1R1 0µg MCA	1R1 10µg MCA	1A1 0µg MCA	1A1 10µg MCA	Carcinogenic Ratio*** 1R1	1A1
1 (St.Mat.)	100.00	10.0	0	50	0	15	4.0	1.2
2 (Rec.Mat.)	97.16	10.0	0	50	0	20	4.0	1.6
3 (B_Ia)	.99	5.0	0	47	0	25	2.7	2.0
4 (B_Ib)	.39	5.0	0	N.D.	0	50	N.D.	4.0
5 (B_E)	8.10	0.5	0	50	0	15	4.0	1.2
6 (B_W)	2.79	2.5	0	36	0	10	2.9	0.8
7 (WA_I)	6.52	10.0	0	50	0	25	4.0	2.0
8 (WA_E)	7.50	10.0	0	5	0	20	0.4	1.6
9 (SA_I)	1.77	5.0	0	30	0	20	2.4	1.6
10 (SA_E)	3.30	10.0	0	40	0	15	3.2	1.2
11 (SA_W)	40.50	10.0	0	32	0	10	2.6	0.8
12 (N_{MeOH})	4.50	10.0	0	63	0	40	5.0	3.2
13 (N_{CH})	18.10	10.0	0	75	0	15	6.0	1.2
14 (N_{MN})	2.70	10.0	0	41	0	5	3.3	0.4

Controls:

trioctanoin:beeswax	0/46	0% tumor incidence at 65 wks.
10µg MCA in trioc:BW	0/46	0% tumor incidence at 64 wks.
10µg MCA/trioctanoin	19/50	38% tumor incidence at 65 wks.
10µg MCA into trioctanoin:beeswax	6/48	13% tumor incidence at 65 wks.
150µg MCA/trioctanoin	13/13	100% tumor incidence at 20 wks.

*Smoke Condensate Fractions Prepared by Dr. A.R. Patel, Meloy Labs, Inc.

**Dose was dependent on toxicity of material given subcutaneously

***Carcinogenic Ratio = $\frac{\%\text{ Tumors Induced with Fraction + 10µg MCA}}{\%\text{ Tumors Induced with 10µg MCA}}$

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CTR-1B: Carcinogenic and Co-Carcinogenic Studies with Cigarette Smoke Condensate (CSC) Inoculated Subcutaneously in C3H/fMai Mice.

Objective:

To determine the carcinogenic and co-carcinogenic properties of various cigarette smoke condensates (CSC) in C3H/fMai mice when administered subcutaneously.

Procedure:

Various samples of CSC from Meloy Labs were diluted 1:5 (w/v) in a 1:1 mixture of trioctanoin:beeswax and inoculated subcutaneously in C3H/fMai mice. These condensates were tested with and without 10 µg MCA delivered at the same site.

Progress:

1. Wax pellets of cigarette smoke condensates were given subcutaneously to mice with and without 3-methylcholanthrene. These mice have currently been on test about 58 weeks (see attached Table 1.).

2. No tumors have been observed among mice that received only cigarette condensates. However, condensates plus MCA (co-carcinogenic) gave tumor incidences that ranged from 0% (condensate #57) to 67% (condensate #60). The mean latency period for tumors in cocarcinogenic mice ranged from 17 weeks (condensate #61) to 48 weeks (condensate #55).

CTR-1B: Carcinogenic and Co-Carcinogenic Studies with Cigarette Smoke Condensates Inoculated Subcutaneously in C3H/fMai Mice

Condensate Tested[a]	Dose (mg)	± MCA[b] 10 µg	Wks. on Test	Tumor Incidence[c]		Latency Period[d]
40	10	-	59	0/7	0	-
		+	59	2/14	14	29.5
41	10	-	59	0/18	0	-
		+	59	6/14	42	32.8
42	10	-	59	0/11	0	-
		+	59	7/13	54	32.0
51	10	-	59	0/8	0	-
		+	59	1/6	16	48
52	10	-	59	0/13	0	-
		+	59	3/19	16	36.3
53	10	-	59	0/15	0	-
		+	59	5/17	29	31.3
54	10	-	59	0/13	0	-
		+	59	6/20	30	32.3
55	10	-	58	0/19	0	-
		+	58	2/19	11	19
56	10	-	58	0/20	0	-
		+	58	7/19	37	38.6
57	10	-	58	0/11	0	-
		+	58	0/8	0	-
58	10	-	58	0/17	0	-
		+	58	6/18	33	34.2
59	10	-	58	0/16	0	-
		+	58	5/19	26	36.4
60	10	-	58	0/8	0	-
		+	58	6/9	67	40.0
61	10	-	58	0/12	0	-
		+	58	1/8	13	17
62	10	-	58	0/15	0	-
		+	58	6/17	35	39.3
57	5	-	57	0/5	0	-
		+	57	3/10	30	31.3
60	5	-	57	0/9	0	-
		+	57	2/6	33	33.5
61	5	-	57	0/6	0	-
		+	57	2/10	20	33.5
10µg MCA/.05 ml trioc		+	65	19/50	38	39 wks.
10µg MCA/.05 ml trioc into BW:T pellet		+	65	6/48	13	46 wks.
10µg MCA/.05 ml BW:T		+	64	0/46	0	-
150µg MCA/.05 ml trioc		+	20	13/13	100	14 wks.
150µg MCA/.05 ml BW:T		+	64	12/20	60	24 wks.

a,b,c,d See footnotes for CTR-1, Table 1.

August 19, 1974

CTR-2: Potential Carcinogenic Effect of Nitrosamines in C57BL/6Cum mice.

Objective:

Preliminary studies were carried out to determine the toxicity of various nitrosamines and their carcinogenicity in C57BL/6Cum mice.

Procedure:

Intraperitoneal (IP) inoculation of newborn C57BL/6Cum mice for acute toxicity and carcinogenicity.

Progress:

1. This experiment has been a pilot study to determine the toxicity and potential carcinogenicity of various levels of nitrosamines in newborn C57BL/6Cum mice. This experiment has been terminated after 12 months testing and the available results are presented in the following tables.

Table 1. Effects of nitrosamines on lung histology.
Table 2. Effects of nitrosamines on liver.
Table 3. Liver histology at 12 months after nitrosamine exposure.

Toxicity effects of various doses of nitrosamines were reported in the early June update summary. Few mice survived for necropsy, but those that did have been evaluated and the results are shown in the accompanying tables.

2. This experiment (CTR-2) has been repeated as CTR-2A which has now been on test about 39 to 49 weeks. Gross and histopathological observations will be performed at 12 or 15 months on these animals.

CTR-2 Table 1: Effects of Nitrosamines on Lung Histology

Material[a] Tested	Diagnosis	Months after Exposure 6	10-11	12
dimethyl-nitrosamine .05 mg	BA lesion	3/23(13%)[b]	0/13(0%)	2/25(8%)
	adenomata	1/23(4%)	4/13(31%)	5/25(20%)
	hyperplasia	0/23(0%)	0/13(0%)	n.a.
	inflamation	0/23(0%)	0/13(0%)	2/25(8%)
	pneumonia	1/23(4%)	1/13(8%)	2/25(8%)
	lymphocyte accum.	n.a.	n.a.	
diethyl-nitrosamine .01 mg	BA lesion	1/36(3%)	0/14(0%)	1/24(4%)
	adenomata	0/36(0%)	4/14(29%)	8/24(33%)
	hyperplasia	1/36(3%)	0/14(0%)	n.a.
	inflamation	0/36(0%)	0/14(0%)	0/24(0%)
	pneumonia	0/36(0%)	2/14(14%)	0/24(0%)
	lymphocyte accum.	n.a.	n.a.	3/24(12%)
dibutyl-nitrosamine 40 mg	BA lesion	10/26(39%)		2/53(4%)
	adenomata	1/26(4%)		13/53(25%)
	hyperplasia	0/26(0%)		n.a.
	inflamation	0/26(0%)		2/53(4%)
	pneumonia	4/26(15%)		8/53(15%)
	lymphocyte accum.	n.a.		5/53(9%)
N-nitroso-piperidine .05 mg	BA lesion	1/16(6%)	3/13(23%)	0/33(0%)
	adenomata	0/16(0%)	1/13(8%)	6/33(18%)
	hyperplasia	2/16(13%)	0/13(0%)	n.a.
	inflamation	0/16(0%)	0/13(0%)	0/33(0%)
	pneumonia	2/16(13%)	0/13(0%)	1/33(3%)
	lymphocyte accum.	n.a.	n.a.	2/33(6%)
N-nitroso-pyrrolidine .13 mg	BA lesion	2/26(8%)	2/13(15%)	1/22(5%)
	adenomata	0/26(0%)	0/13(0%)	2/22(9%)
	hyperplasia	2/26(8%)	1/13(8%)	n.a.
	inflamation	0/26(0%)	2/13(16%)	0/22(0%)
	pneumonia	3/26(12%)	0/13(0%)	0/22(0%)
	lymphocyte accum.	n.a.	n.a.	0/22(0%)
Trioctanoin .05 ml	BA lesion		1/4(25%)	1/12(8%)
	adenomata		0/4(0%)	0/12(0%)
	hyperplasia		0/4(0%)	0/12(0%)
	inflamation		0/4(0%)	0/12(0%)
	pneumonia		0/4(0%)	0/12(0%)
	lymphocyte accum.		n.a.	n.a.

[a] Various nitrosamines dissolved in trioctanoin and given by intraperitoneal injection to newborn C57BL/6Cum mice.

[b] Number of mice positive divided by number examined.

CTR-2 Table 2: Effects of Nitrosamines on Liver

Material[a] Tested	Liver Status		Months After Exposure 6		10		11		12	
dimethylnitrosamine	abnormal[b]	8/12	(67%)				11/12	(85%)	9/12	(75%)
.05 mg	tumored[c]	5/12	(42%)				4/13	(31%)	8/12	(67%)
diethylnitrosamine	abnormal	2/24	(8%)	2/15	(13%)				6/24	(25%)
.01 mg	tumored	2/24	(8%)	0/15	(0%)				3/24	(13%)
dibutylnitrosamine	abnormal	2/26	(8%)						22/53	(42%)
.40 mg	tumored	0/26	(0%)						14/53	(26%)
N-nitrosopiperidine	abnormal	1/6	(17%)	2/13	(15%)				3/33	(9%)
.05 mg	tumored	0/6	(0%)	0/13	(0%)				0/33	(0%)
N-nitrosopyrrolidine	abnormal	1/14	(7%)	0/13	(0%)				1/22	(5%)
.13 mg	tumored	0/14	(0%)	0/13	(0%)				0/22	(0%)
Trioctanoin	abnormal						0/4	(0%)	1/8	(13%)
.05 ml	tumored						0/4	(0%)	0/8	(0%)

[a]Various nitorsamines given by intraperitoneal injection to newborn C57BL/6Cum mice.

[b]Number of abnormal livers (includes tumors, discolorations and lesions) divided by number of livers examined.

[c]Includes only livers with tumors divided by number of livers examined.

August 19, 1974

CTR-2 Table 3: Effect of Nitrosamines* on Liver Histology at 12 Months

Pathology	Trioc .05 ml	DMN** .05 mg	DEN .01 mg	PIP .05 mg	PYR .13 mg	DBN .40 mg
Centrilobular congestion-fatty change	1/12 8%	-	1/24 4%	11/33 33%	4/22 18%	13/33 25%
Hyalin droplet degeneration	-	-	6/24 25%	3/33 9%	4/22 18%	17/53 32%
Hepatoma	-	-	2/24 8%	-	-	-
Nodular hyperplasia	-	-	2/24 8%	1/33 3%	1/22 5%	14/53 27%
Perivascular hymphocyte accumulation	-	-	2/24 8%	1/33 3%	-	-
Lymphocyte leukemia	-	-	1/24 4%	-	1/22 5%	-
Reticular cell neoplasm	-	-	-	1/33 3%	-	3/53 6%
Lymphocytic neoplasm	-	-	-	-	-	1/53 2%

* Nitrosamines given by intraperitoneal injection to newborn C57BL/6Cum mice.

** dimethylnitrosamine (DMN) - awaiting pathology.

diethylnitrosamine (DEN)

dibutylnitrosamine (DBN)

N-nitrosopiperidine (PIP)

N-nitrosopyrrolidine (PYR)

trioctanoin (trioc)

August 19, 1974

CTR-2A: Potential Carcinogenic Effect of Nitrosamines in C57BL/6Cum Mice.

Objective:

To test the carcinogenicity of several nitrosamines in newborn C57BL/6Cum mice.

Procedure:

Intraperitoneal (IP) inoculation of newborn C57BL/6Cum mice for acute toxicity and carcinogenicity.

Progress:

1. Mice have been on test for periods that range from 42 to 52 weeks. Mortality has been greatest with dimethylnitrosamine (48%), diethylnitrosamine (46%), and dibutylnitrosamine (42%). Female mice, particularly with these substances, appear more susceptible to death than the males.

2. No tumors or other unusual findings have been observed with the mice on test. Necropsy will be performed on mice at 14 months on test.

CTR-2A August 19, 1974

Table 1. Survival Incidence of Mice Exposed to Nitrosamines

Material[a] Tested	Sex	# Mice Tested	# Mice Survive	Mortality	Wks. on Test
dimethylnitrosamine .05 mg	♂	40	22	45%	52
	♀	40	21	48%	52
diethylnitrosamine .01 mg	♂	51	42	18%	51
	♀	51	31	46%	51
N-nitrosopiperidine .05 mg	♂	31	26	26%	51
	♀	22	15	32%	51
N-nitrosopyrrolidine .13 mg	♂	47	24	28%	50
	♀	48	43	10%	50
dibutylnitrosamine .40 mg	♂	49	33	33%	50
	♀	48	28	42%	50
trioctanoin .05 ml	♂	12	10	17%	46
	♀	13	9	31%	46
trioctanoin .05 ml	♂	10	10	0%	42
	♀	10	10	0%	42

[a] Nitrosamines dissolved in trioctanoin and injected intraperitoneally in newborn C57BL/6Cum mice.

CTR-3 (W-204) August 19, 1974

Induction of Squamous Cell Carcinoma in the Respiratory Tract of Mice by Intratracheal Instillation of MCA.

Objectives:

Induction of squamous cell carcinomas in mice by intratracheal instillation of MCA as described by Nettesheim for the BC3F1 mice. This study was expanded to include the parent strains of this hybrid.

Procedure:

Four mouse strains (C57BL/6Cum, C57BL/Cum, C3H/AnfCum and BC3F1/Cum) have been treated intratracheally with gelatin vehicle or 500µg MCA in gelatin. Mice were either given 1 treatment or 3 treatments or 6 treatments at weekly intervals. The gelatin controls all received 6 treatments.

We experienced difficulty in retaining the MCA in suspension and obtained wide variations in the dose administered. To correct this problem we sonicated extensively the MCA just prior to inoculation. In light of the poor tumor induction we obtained and results presented at the recent Seattle lung carcinogenesis meeting we feel we may have actually reduced the particle size of the MCA to such a fine level that we did not obtain as many tumors as Dr. Nettesheim's group did with less sonication. Early results in additional studies indicate this may be the case.

Our method of describing time on test is different than Nettesheim's time on test. Since we treated mice 1x, 3x and 6x, we found it easier to describe time on test based on 1st treatment. Nettesheim reported his data on the basis of time after 6th treatment, therefore, there is a six week difference in time on test from that in our experiments.

Progress:

1. We lost numerous mice due to pneumonia during the inoculation period (table 1). Since few mice were lost in the gelatin vehicle control group it was concluded that the corrosive nature and possibly the immunosuppressive effects of MCA may have contributed to the high incidence of pneumonia. The number of animals dying during the injection period increased in proportion to the number of injections. With 1 instillation 2-22% died, with 3 treatments 30-47% died and with 6 treatments 45-94% died. The C57BL/Cum was the most susceptible while the other strains presented approximately similar pictures. (see table 1)

Some of this pneumonia was due to Sendai as demonstrated in table 2. We apparently introduced Sendai into the lab with the groups D,E & F. These results would indicate the C3H/f was the most resistant.

2. Mice were test sacrificed at varying intervals after inoculation for gross and histological examination. This is reflected by the number of paths taken between the 7th and 30th weeks in table 3. Most mice have now been on test in excess of 70 weeks, therefore we are in the process of terminating the experiment. The results so far indicate that 1 treatment is not sufficient and 6 treatments on a weekly basis leads to too many early deaths. The number of squamous cell carcinomas has been disappointingly low. It would appear however that more squamous cell carcinomas have occurred in the C3H than the other strains. The highest incidence of other lung tumors has been in the BC3F1. The highest number of BA lesions have occurred in the C3H/f and the C57BL mice. Any definitive differences will have to wait until all mice have been sacrificed and histopathological diagnoses made.

3. Tumors and BA lesions have been transplanted into new born mice. We have had successful transplants of squamous carcinomas, keratinized BA lesions, BA lesions with alveolar adenomas in the same lung, and alveolar adenocarcinomas. These studies are still in progress. See tables 4 and 5.

Conclusions:

1. This experiment has not provided the tumor incidence expected based on Nettesheim's report, however it has provided experience in this technique of inoculation.

2. We have also concluded that the particle size of the MCA is a significant factor and future experiments will be performed without extensive sonication of the carcinogen.

3. The dose level of 500µg at weekly intervals is too great and both dose levels and intervals between injections are being investigated.

4. Familiarity with the various lung tumors and lesions has been obtained by our pathologist, Dr. B. Sass. He has collaborated with Dr. Robert M. Kovatch of San Francisco, Dr. Stewart of NCI and Mr. William Blair of Chicago and they are in agreement as to the histopathological diagnoses. Dr. Sass is at present attempting to improve his staining technique to provide better diagnosis.

W-204, CTR-3

Table 1. Intratracheal Inoculation of mice with MCA. Losses during treatment period i.e., 1x,3x,6x MCA or gelatin.

Mouse Strain	Group #	Treatment	Date Initiated (1973)	Initial # mice	Losses During Treatment Weeks Observation 1x 0	2x 1	3x 2	4x 3	5x 4	6x 5	6	# mice after final Rx
C3H/Anf ♂	IA	gel 6x	3/19	27				1				26
	IB	500 MCA 1x	3/26	48	4							44
	IC	500 MCA 3x	3/22	40	2	8	4					26
	IE	500 MCA 3x	4/27	50	1	5	12					32
	IF	500 MCA 3x	6/4	65		2	1					62
	ID	500 MCA 6x	3/20	60		5	5		3	3		44
C57BL/6 Cum ♂	IIA	gel 6x	3/20	30			2		2			26
	IIB	500 MCA 1x	3/30	51								51
	IIC	500 MCA 3x	3/22	45		2	3					40
	IIE	500 MCA 3x	4/12	50			6					44
	IIF	500 MCA 3x	5/7	61		6	16					39
	IID	500 MCA 6x	3/21	57		1	1	4	4	2		45
BC3F1/Cum ♂	IIIA	gel 6x	3/19	30					1			29
	IIIB	500 MCA 1x	3/30	50	1							49
	IIIC	500 MCA 3x	3/22	35	2	8	3					22
	IIIE	500 MCA 3x	4/11	55	1	4	8					42
	IIIF	500 MCA 3x	5/8	62			4					58
	IIID	500 MCA 6x	3/20	64		6	8	7	16	2		25
C57BL/Cum ♀	IVA	gel 6x	4/16	30				2	1	5		22
	IVB	500 MCA 1x	4/19	50								50
	IVC	500 MCA 3x	4/19	50		2	4					44
	IVE	500 MCA 3x	4/27	80		3	35					42
	IVF	500 MCA 3x	5/8	66			1					65
	IVD	500 MCA 6x	4/18	50		3	3	6	6	8		24

CTR-3 (W-204) Table 2. Presence of Sendai Antibody* in sera taken at monthly intervals and at the time mice were sacrificed for histopathology.

		Mouse Strain							
		C3H/Anf		C57BL/6		BC3F1		C57BL	
Group #	Treatment	P/T**	%	P/T	%	P/T	%	P/T	%
A	6x gelatin	0/15	0	1/16	6	0/17	0	1/18	6
B	1x MCA	0/27	0	0/30	0	0/30	0	0/30	0
C	3x MCA	0/15	0	0/24	0	0/10	0	0/30	0
E	3x MCA	2/17	12	0/28	0	32/37	86	19/36	53
F	3x MCA	0/28	0	17/25	68	13/42	31	13/43	30
D	6x MCA	2/29	7	9/27	33	0/16	0	11/18	61

* Sendai antibody was detected primarily during the 1st and second month the mice were on test.

** # positive mice/total number tested.

These results would indicate that the initial experimental animals in groups A, B, C, Dwere generally less affected. The exception was the C57BL/6 & the C57BL group D's and was due to the extended treatment period which overlapped the treatment periods of groups E and F. The spread of Sendai was limited since we isolated the sick animals as quickly as possible.

9/25/73

CTR-3. (W-204)

Table 3. Histopathology on Lungs from Mice Treated Intratracheally with Gelatin on 500µg MCA in Gelatin at 10 Weeks of Age.

Mouse Strain / Histopathology	6x Gel 7-20	21-30	51-60	61-70	T	1x MCA 7-20	21-30	31-40	41-50	51-60	61-70	71-80	T
C3H/AnfCum													
# Paths	4	0	5	5	14	0	0	0	0	7	1	0	8
Negative	3		3	5	11					3	1		4
Pneumonia	1		0	0	1					0	0		0
BA Lesions	0		1	0	1					0	0		0
Tumors	0		0	0	0					3	1		4
Sq. c.c.	0		0	0	0					0	0		0
C57BL/6Cum													
# Paths	2	0	0	0	2	4	4	2	0	5	0	0	15
Negative	2				2	4	2	2		0			8
Pneumonia	0				0	0	0	0		1			1
BA Lesions	0				0	0	0	0		1			1
Tumors	0				0	0	2	0		2			4
Sq. c.c.	0				0	0	0	0		0			0
C57BL/Cum													
# Paths	13	0	2	0	15	6	0	1	8	2	0	0	17
Negative	13		1		14	6		1	0	1			8
Pneumonia	0		0		0	0		0	0	1			1
BA Lesions	0		1		1	0		0	2	0			2
Tumors	0		0		0	0		0	2	0			2
Sq. c.c.	0		0		0	0		0	0	0			0
BC3F1/Cum													
# Paths	0	3	1	0	4	0	0	0	0	5	0	0	5
Negative		1	1		2					1			1
Pneumonia		1	1		2					1			1
BA Lesions		1	0		1					2			2
Tumors		0	0		0					4			4
Sq. c.c.		0	0		0					0			0

Paths = Number of Pathologies taken
Sq. c.c.= Squamous cell carcinomas
B.A. = Broncho-alveolar lesions
T = Total

CTR-3, (W-204)

Contd.- Table 3. Histopathology on Lungs from Mice Treated Intratracheally with Gelatin on 500µg MCA in Gelatin at 10 Weeks of Age.

Mouse Strain / Histopathology	3x MCA 7-20	21-30	31-40	41-50	51-60	61-70	71-80	T	6x MCA 7-20	21-30	31-40	41-50	51-60	61-70	71-80	T
C3H/AnfCum																
# Paths	21	24	1	16	6	0	0	66	13	14	2	0	7	7	6	43
Negative	13	16	0	0	1			30	11	5	1		0	0	0	17
Pneumonia	0	1	0	2	0			3	0	0	0		2	1	0	3
BA Lesions	8	3	1	3	1			16	1	5	0		3	6	0	15
Tumors	1	4	1	12	4			20	0	4	1		6	7	0	18
Sq. c.c.	4	1	1	2	1			8	1	0	1		2	0	0	4
C57BL/6Cum																
# Paths	21	25	8	11	2	1	0	68	18	12	0	3	1	0	0	34
Negative	16	15	3	1	0	0		35	11	6		0				17
Pneumonia	3	4	3	3	2	1		16	3	2		1	1			7
BA Lesions	3	6	3	6	1	0		19	3	3		2	1			9
Tumors	1	2	3	5	2	1		14	2	2		1	1			6
Sq. c.c.	0	0	3	2	0	0		5	0	0		1	0			1
C57BL/Cum																
# Paths	57	20	4	24	2	0	0	107	26	0	0	1	1	1	0	29
Negative	32	11	2	0	1			46	15			0	1	1		17
Pneumonia	8	3	0	6	0			17	8			0	0	0		8
BA Lesions	12	6	1	19	1			39	2			1	0	0		3
Tumors	4	0	0	13	0			17	0			1	0	0		1
Sq. c.c.	1	0	1	2	0			4	2			0	0	0		2
BC3F1/Cum																
# Paths	13	11	0	16	14	1	0	54	12	6	0	1	5		0	24
Negative	9	6		1	0	0		16	6	2		0	0			8
Pneumonia	0	4		4	3	0		12	5	1		0	3			9
BA Lesions	3	2		9	6	0		20	1	3		0	3			7
Tumors	0	0		13	12	0		25	2	1		1	6			10
Sq. c.c.	0	1		3	4	1		8	0	0		0	0			0

Paths = Number of Pathologies taken

Sq. c.c. = Squamous cell carcinomas.

B.A. = Broncho-alveolar Lesions

T = Total

CTR-3, (W-206,W-207,W-208,W-209)

Table 4. Successful Lung Tumor Transplants in Various Strains of Mice.

Mouse Strain	Rx	MCA (µg)	# days on test	Path Diagnosis	Days Required to Reach 1cm Transplant P1*	P2	P3
C3H/f	6x	500	382	Squamous cell carcinoma	29	30	51
C3H/f	6x	500	404	Alveolar adenoma	35	57	
C3H/f	3x	500	350	-	80		
C57BL/6	3x	500	259	BA lesions, Keratinz.	45	97	
C57BL/6	6x	500	342	Squamous cell carcinoma	23		
C57BL/6	3x	500	358	Squamous cell carcinoma	80		
C57BL/6	3x	500	365	BA lesions, Alve. adenoma	45	37	
C57BL/6	3x	500	362	Alveo. adenocarcinoma	34	55	
BC3F1	3x	500	311	Adenoma, Carcinoma	91		
BC3F1	6x	500	376	Alveo. Adenocar., pneu.	85		
BC3F1	6x	500	376	Alveolar ademona	83		
BC3F1	1x	500	386	Alve. Adenoma, Alveolar adenocarcinoma	59	53	
BC3F1	3x	500	357	Alve. adenocar., BA les.	100		
BC3F1	3x	500	384	Alve. carcinoma, Alv. adenoma	100	53	
BC3F1	3x	500	384	Alve.Adenoma, car. of pleura	13	30	57
BC3F1	3x	500	357	Sq. cell car.,Alv.aden.	43		
BC3F1	3x	500	365	Squamous metastasis	35	57	
BC3F1	3x	500	397	-	87		
C57BL	3x	500	304	Papill. scirrhus, Sq. cell carcinoma	59	37	

* P = Passage #

CTR-3. (W-206)

Table 5. Unsuccessful Lung Tumor Transplants Held for Four Months

Mouse Strain	Rx	MCA (µg)	# Days on Test	Path Diagnosis
C3H/f	3x	500	284	BA Lesions
C3H/f	3x	500	284	BA Lesions, Fibrosis
C3H/f	6x	Gel	371	Essentially normal
C3H/f	6x	Gel	371	Hyperplasia of spleen
C3H/f	6x	Gel	371	1 mild BA lesion
C3H/f	6x	Gel	371	Essentially normal
C3H/f	6x	Gel	371	Essentially normal
C3H/f	1x	500	376	Lungs normal
C3H/f	6x	500	382	Broncho-Alveolar Adenoma, BA Lesions
C3H/f	6x	500	392	BA Lesions (mild)
C3H/f	1x	500	386	Essentially normal
C3H/f	3x	500	376	Essentially normal
C3H/f	6x	500	404	Alveolar carcinoma, bronch. epith. hyperplasia
C3H/f	6x	500	426	Path not available at this time.
C3H/f	1x	500	420	
C3H/f	6x	500	426	
C3H/f	6x	500	426	
C3H/f	6x	500	426	
C3H/f	3x	500	350	

CTR-3, (W-207, W-208)

Contd.- Table 5. Unsuccessful Lung Tumor Transplants Held for Four Months.

Mouse Strain	Rx	MCA (µg)	# Days on Test	Path Diagnosis
C57BL/6	6x	500	342	BA Lesions
C57BL/6	1x	500	357	Normal
C57BL/6	1x	500	363	Essentially normal
C57BL/6	1x	500	363	Interstitial pneumonia
C57BL/6	3x	500	337	Severe BA Lesions
C57BL/6	1x	500	362	Mild BA Lesions
BC3F1	1x	500	372	Alveolar adenoma, adenocarcinoma
BC3F1	1x	500	372	Alveolar adenoma
BC3F1	6x	500	382	Alveolar adenocarcinoma
BC3F1	3x	500	333	Sq. cell carcinoma, Alveolar adenoma
BC3F1	3x	500	370	Moderate BA Lesions
BC3F1	3x	500	370	Alveolar adenoma, BA Lesions
BC3F1	3x	500	343	Essentially normal
BC3F1	3x	500	384	Alveolar Adenoma, BA Lesions
BC3F1	3x	500	357	Interstitial pneu., broncho-adenoma
BC3F1	1x	500	396	Essentially normal
BC3F1	6x	500	414	Alveolar adenocarcinoma, BA Lesions
BC3F1	6x	Gel	415	Essentially normal
BC3F1	3x	500	365	Adenocarcinoma

CTR-3, (W-208, W-209)

Contd - Table 5. Unsuccessful Lung Tumor Transplants Held for Four Months.

Mouse Strain	Rx	MCA (µg)	# Days on Test	Path Diagnosis
C57BL	3x	500	337	Alveolar Adenoma, BA Lesions
C57BL	3x	500	318	Alveolar Adenoma, BA Lesions
C57BL	6x	Gel	345	Normal
C57BL	6x	Gel	345	Moderate BA Lesions
C57BL	1x	500	342	BA Lesions, Alveolar Adenoma
C57BL	1x	500	342	Essentially normal
C57BL	1x	500	342	1 Alveolar Adenoma
C57BL	1x	500	342	Essentially normal
C57BL	1x	500	342	Mild BA lesions
C57BL	1x	500	342	Essentially normal
C57BL	1x	500	342	Essentially normal
C57BL	1x	500	342	Essentially normal
C57BL	3x	500	343	Alveolar Adenoma, BA Lesions
C57BL	3x	500	343	Squamous cell carcinoma
C57BL	3x	500	343	Alveolar Adenoma, BA Lesions, pneumonia
C57BL	6x	500	344	Alveolar adenoma, BA Lesions
C57BL	3x	500	343	Squamous cell carcinoma
C57BL	3x	500	336	BA lesions
C57BL	3x	500	336	BA lesions

CTR-3A (CW048) August 19, 1974

Induction of Squamous Cell Carcinoma in the Respiratory Tract of Mice by Intratracheal Instillation of MCA.

Objectives:

Further studies with MCA intratracheal instillation for the induction of squamous cell carcinomas. These studies have compared the use of gelatin and trioctanoin as the vehicle and the use of treatment schedules of once a week vs. once every two weeks.

Procedure:

C3H/fMai, male and female mice have been given either 500µg MCA in gelatin or 250µg MCA in trioctanoin, once a week or once every two weeks. Our original intention was to give 500µg in each diluent, however MCA was not soluble at this level in the .02ml used for IT inoculations.

Progress:

1. These studies with the MCA given weekly were terminated at 15 weeks while the rest of the study is in the 33rd week after the initial dose of MCA. The survival rate, during the treatment period, of mice receiving 500µg MCA in gelatin at weekly intervals was only 9-36%. When MCA in gelatin was given every other week 43-48% of the mice survived. Mice treated with 250µg MCA in trioctanoin survived significantly better with almost as many control mice dying as the MCA treated mice. (see table 1)

2. The mice receiving MCA in gelatin at biweekly intervals have died or had to be sacrificed at a significantly greater rate than those receiving MCA in trioctanoin. Histological examination of five of these mice has demonstrated that all had squamous cell carcinomas. See table 2.

Comments:

This experiment very quickly demonstrates that possibly the dose of MCA given was toxic and that possibly survival rate might be improved by lower doses at 2 week intervals. Early histological evidence indicates that squamous cell carcinomas can be produced in approximately 30 weeks after the initial 500µg MCA instillation in gelatin on a 1x/week basis.

CTR-3A Table 1

Effects of schedule, vehicle, and dose of MCA given intratracheally on survival of C3H/f male and female mice

Treatment			Sex	Number of mice on test		33 wk. survival results	
µg MCA	Vehicle	Schedule		# of mice after 6Rx/ initial # of mice	% Surv.	# of mice surv./ # of mice on test	% Surv.
0	gelatin	1x/week	♂	24/25	96%	--- Terminated	
			♀	21/25	84	--- at 17 weeks	
500	gelatin	1x/week	♂	7/75	9	--- due to few	
			♀	9/25	36	--- surviving mice	
0	gelatin	1x/2 wks	♂	24/25	92	23/23.	100%
			♀	24/25	96	24/24	100
500	gelatin	1x/2 wks	♂	32/75	43	3/32	9
			♀	12/25	48	12/12 at 24 wks.	100
0	trioc.	1x/week	♂	24/25	96	23/24	96
			♀	19/25	76	18/19	95
250	trioc.	1x/week	♂	54/75	72	49/54	91
			♀	20/25	80	12/20	60
0	trioc.	1x/2 wks	♂	10/25	40	9/10	90
			♀	15/25	60	15/15	100
250	trioc.	1x/2 wks	♂	52/75	69	48/52	77
			♀	19/25	76	16/19	84

CTR-3A (C048) Table 2: Histopathology on Lungs from C3H/fMai Mice Treated Intratracheally with MCA in Gelatin or Trioctanoin Sex Times on a Weekly or Biweekly Schedule.

Dose / # Paths	6x (1x/week) Weeks on Test 20- 31- 41- 51- 30 40 50 60	Total	6x (1x/biweekly) Weeks on Test 20- 31- 41- 51- 30 40 50 60	Total
Gelatin				
# Paths				
Negative				
Pneumonia				
BA Lesions				
Tumors				
Sq. c.c.				
Pleural Invas.				
Metastasis				
Trioc				
# Paths				
Negative				
Pneumonia				
BA Lesions				
Tumors				
Sq. c.c.				
Pleural Invas.				
Metastasis				
500ug MCA/Gel				
# Paths			5	5
Negative			0	0
Pneumonia			0	0
BA Lesions			3	3
Tumors			5	5
Sq. c.c.			5	5
Pleural Invas.			5	5
Metastasis			0	0
250ug MCA/Trioc				
# Paths				
Negative				
Pneumonia				
BA Lesions				
Tumors				
Sq. c.c.				
Pleural Invas.				
Metastasis				

28A.

CTR-3B (CW052) August 19, 1974

Induction of Squamous Cell Carcinoma in the Respiratory Tract of Mice by Intratracheal Instillation of MCA.

Objectives:

To repeat CTR-3A using MCA in gelatin and trioctanoin, given at weekly and biweekly intervals in C57BL/6Cum mice.

Procedure:

C57BL/6Cum male mice were used for this study since female mice were not available. 500µg MCA in gelatin or 250µg MCA in trioctanoin was given at weekly or biweekly intervals. Due to the high incidence of deaths in mice treated with 500µg MCA in gelatin at weekly intervals only 5 treatments were given. All other mice received 6 treatments.

Progress:

1. This study is in the 33rd week after the initial treatment. No evidence of respiratory difficulty is seen in the mice remaining from the weekly MCA-gel treatments while the biweekly treated mice appear sick and deaths are occurring. Mice given MCA in trioctanoin are healthy.

2. The initial deaths and the surviving mice at this time are seen in table 1.

3. Histopathological studies on 22 sick mice, 27-29 weeks after the initial inoculation (13-17 weeks after the 6th biweekly injection), have demonstrated squamous cell carcinomas in all mice. We will now start to test sacrifice mice for additional indications of tumor induction.

CTR-3B Table 1

Effects of schedule, vehicle, and dose of MCA given intratracheally on survival of C57BL/6 mice

Treatment			Number of mice on test		33 week survival results	
µg MCA	Vehicle	Schedule	# of mice after Rx/ initial # of mice	% Surv.	# of mice surv./ # of mice on test	% Surv.
0	gelatin	6x/1x wk.	43/45	93%	42/43	98%
500	gelatin	5x/1x wk.	36/100	36	24/36	67
0	gelatin	6x/1x Biweekly	49/50	98	48/49	98
500	gelatin	6x/1x Biweekly	62/100	62	26/36	72
0	trioc.	6x/1x wk.	45/50	90	45/45	100
250	trioc.	6x/1x wk.	78/100	78	78/78	100
0	trioc.	6x/1x Biweekly	45/90	90	45/45	100
250	trioc.	6x/1x Biweekly	89/100	89	86/89	97

CTR-3A (C052) Table 2: Histopathology on Lungs from C57BL/6 Mice Treated Intratracheally with MCA in Gelatin or Trioctanoin 6 Times on a Weekly or Biweekly Schedule.

Dose / # Paths	6x (1x/week) Weeks on Test 20-30	31-40	41-50	51-60	Total	6x (1x/biweekly) Weeks on Test 20-30	31-40	41-50	51-60	Total
Gel										
# Paths										
Negative										
Pneumonia										
BA Lesions										
Tumors										
Sq. c.c.										
Pleural Invas.										
Metastasis										
Trioc										
# Paths										
Negative										
Pneumonia										
BA Lesions										
Tumors										
Sq. c.c.										
Pleural Invas.										
Metastasis										
250.0ug MCA/Trioc										
# Paths						1				1
Negative						1				1
Pneumonia						0				0
BA Lesions						0				0
Tumors						0				0
Sq. c.c.						0				0
Pleural Invas.						0				0
Metastasis						0				0
500.0ug MCA/Gel										
# Paths						21				21
Negative						0				0
Pneumonia						0				0
BA Lesions						11				11
Tumors						20				20
Sq. c.c.						21				21
Pleural Invas.						19				19
Metastasis						2				2

CTR-3C (CW055) August 19, 1974

Induction of Squamous Cell Carcinoma in the Respiratory Tract of Mice by Intratracheal Instillation of MCA.

Objectives:

Based on the two previous studies with MCA intratracheal injection it would appear that smaller doses must be used to allow the mice to survive the inoculation period; therefore this experiment was undertaken to determine if tumor induction could be obtained with minimal doses.

Procedure:

C3H/f female mice were injected with either 62.5, 125 or 250 µg MCA in gelatin at weekly intervals for 6 and 12 times.

Progress:

1. The mice in this study are now on for 28 weeks after the initial MCA treatment.

2. The deaths through these treatment schedules and during the following observation period have significantly been reduced. (see table 1)

3. Seven mice have been autopsied 21-23 weeks after initial MCA injection (total of 12 treatments) and all were found to have squamous cell carcinomas. (see table 2)

CTR-3C Table 1

Effects of dose and number of times of IT administered on the survival of C3H female mice

Number of treatments	MCA dose (μgm)	Number of mice on test		28 week survival results	
		# of mice after final Rx/initial # of mice	% Surv.	# of mice dead/ # of mice on test	% Surv.
6	0	24/25	96	24/24	100
	62.5	47/50	94	42/47	89
	125.0	49/50	98	48/49	98
	250.0	44/50	88	34/44	77
12	62.5	40/47	85	37/40	93
	125.0	38/46	83	33/38	87
	250.0	31/51	61	24/31	77

CTR-3C (C055) Table 2: Histopathology on Lungs from C3H/f Female Mice Treated Intratracheally 6-12 Times with Three Dose Levels of MCA, One Time/Week.

Dose / # Paths	6x (1x/week) Weeks on Test 20-30	31-40	41-50	51-60	Total	12x (1x/week) Weeks on Test 20-30	31-40	41-50	51-60	Total
Gelatin										
# Paths										
Negative										
Pneumonia										
BA Lesions										
Tumors										
Sq. c.c.										
Pleural Invas.										
Metastasis										
62.5ug MCA										
# Paths	1				1	1				1
Negative	1				1	0				0
Pneumonia	0				0	1				1
BA Lesions	0				0	0				0
Tumors	0				0	0				0
Sq. c.c.	0				0	0				0
Pleural Invas.	0				0	0				0
Metastasis	0				0	0				0
125.0ug MCA										
# Paths						3				3
Negative						0				0
Pneumonia						0				0
BA Lesions						2				2
Tumors						2				2
Sq. c.c.						2				2
Pleural Invas.						1				1
Metastasis						0				0
250.0ug MCA										
# Paths	1				1	8				8
Negative	0				0	0				0
Pneumonia	0				0	6				6
BA Lesions	0				0	6				6
Tumors	1				1	7				7
Sq. c.c.	1				1	2				2
Pleural Invas.	0				0	1				1
Metastasis	0				0					

32A.

CTR-3D August 19, 1974

Induction of Squamous Cell Carcinoma in the Respiratory Tract of Mice by Intratracheal Instillation of MCA.

Objectives:

To repeat CTR-3C in male mice to determine if there is a difference in susceptibility between male and female mice.

Procedure:

C3H/f male mice will be inoculated 6-12 times with varying doses of MCA via IT route at weekly intervals.

Progress:

This experiment was initiated August 12, 1974.

CTR-4 (W-200) August 14, 1974

Subcutaneous Treatment of C57BL, C3H/Anf and BC3F1 Mice with 3-Methylcholanthrene for Comparison with Intratracheal Treatment for Tumor Induction.

Objectives:

Several mouse strains were selected in CTR-3 for lung carcinogenesis studies which had not previously been studied in this laboratory by subcutaneous MCA treatment. In order to establish their relative susceptibility with previously tested mouse strains by the same criteria, studies were undertaken using our standard subcutaneous evaluation procedures.

Procedure:

Weanling mice of seven strains were given subcutaneous injections of three doses of MCA to determine relative susceptibility to subcutaneous tumor induction.

Progress:

This study has been completed with the establishment of relative susceptility to MCA given subcutaneously, the AHH inducibility and the presence of group specific (gs) antigens for the type C RNA tumor viruses. (see tables)

Conclusions:

1. The C3H/f mice are the most susceptible of the strains tested to MCA subcutaneous tumorigenesis (table 1). There is probably no significant difference in the C3H/f mice from Microbiological Associates and Cumberland View Farms, although some differences were noted with 37.5µg MCA. The Mai strain has consistently given almost as many tumors with 37.5µg and 150µg. This was the first time we had run three doses with the Cum strain. One of the most significant factors in the high degree of susceptibility of this strain is the short latency period.

2. The C57BL and the C57BL/6 do not show significant differences in MCA tumorigenicity (table 1). This was our first experience with the parent strain - the C57BL.

3. The hybrid mice strains (BC3F1/Cum and B6C3F1/Mai) do not differ significantly in tumor indicence, however the latency is greater in the B6C3F1/Mai mice. We did not test the C57BL/6Mai mice in this study, however previous studies have demonstrated significant lower susceptibility to subcutaneous MCA carcinogenesis in the hybrids than the C57BL/6Cum strain. The Mai strain also has higher levels of gs antigen than the Cum strain. One of the significant findings of this study is the apparent in- increased susceptibility of the hybrid when the B6/Cum was crossed with the C3/Mai and the susceptibility approached

that of the C3H parent. This strain is not commercially available. This strain should be characterized for susceptibility to IT inoculation with a variety of carcinogens.

4. Spleens and tumors have been tested by CF test for gs antigen to determine type C RNA viral genome expression. As seen in Table 2 the C57BL/6 and C57BL mice were virtually negative for gs antigen as previously observed. The C3H/Anf and C3H/f were low in gs antigen at 4 weeks, however, the MCA induced tumors were nearly all positive even at 1:8 dilutions of the 40% tumor sonicate extract. The BC3F1 appeared to take on the same gs antigen expression as the C3H/Anf parent which reflects previous findings with the C57BL/6Mai and C3H/fMai parents. The Mai strain of C57BL/6 has more gs antigen expression than the C57BL/6Cum strain. The B6C3F1/Cum x Mai mice were bred in our laboratory from C57BL/6Cum females and C3H/fMai males. It would appear from the control mice sacrificed at 4 weeks of age to have comparable gs antigen expression as the B6C3F1/Mai, therefore, the C3H/fMai may have contributed the gs antigen expression tendency to the hybrid strains.

5. The AHH inducibility (table 3) of the C57BL, C57BL/6 and BC3F1 mice are all similar and are more inducible than the C3H mice. The significance of differences in the degree of inducibility is not known. It is felt, however, that if an animal is inducible, the initial event of transformation occurs which governs the tumor incidence. The latency of tumor development is probably not dependent on inducibility but rather on other host related factors, as immunocompetence.

6. The BC3F1 mice were also used in CTR-3 and found to be a significantly more sturdy strain than either of the parents. The hybrid has the nice features of the C3H/f in that it is easy to handle, does not fight nor develop skin lesions common to the C57BL/6. The BC3F1 exhibits the barbering character common to approximately 10% of the C57BL parents, however in the BC3F1 barbering occurs in virtually 100% of the mice and is confined to the nose and eye region. In the C57BL/6 mice they also barber the shoulder region.

Comments:

The C57BL/6 strain produces few tumors with DMBA or BP while the C3H/f is highly susceptible. As a further characterization of the hybrid strain these studies have been included in a later study (CTR-19).

CTR-4, (W-200) -- Table 1: Subcutaneous Carcinogenic Effects of MCA in Various Strains of Mice (8 Months Observation)

Mouse Strain	MCA Dose (μg)	Tu/T	%	Latency (wks) Av.	50%	Range	CI	TD_{50} μg MCA
C3H/AnfCum	9.38	9/29	31	20.7	23.2	18-35	21	13
	37.5	16/29	55	17.1	13.8	11-35	46	
	150.0	25/29	86	13.9	10.2	8-23	89	
C3H/fMai	9.38	9/28	32	22.6	18.0	15-35	20	20
	37.5	24/29	83	17.5	14.8	13-33	68	
	150.0	27/30	90	13.4	9.9	9-24	96	
C57BL/Cum	9.38	2/22	9	28.5	27.0	27-30	5	18
	37.5	14/25	56	22.8	20.0	16-30	35	
	150.0	18/25	72	17.5	15.0	13-23	59	
C57BL/6Cum	9.38	3/27	11	22.6	18.0	18-25	7	40
	37.5	12/28	43	21.2	19.5	12-34	29	
	150.0	19/30	63	15.8	15.1	14-27	57	
$BC3F_1$/Cum (C57BL x C3H/Anf)	9.38	3/30	10	21.3	20.5	20-23	7	82
	37.5	8/30	27	20.5	14.0	11-35	19	
	150.0	22/30	73	14.0	12.2	9-24	75	
B6C3F1/Mai (C57BL/6 x C3H/f)	9.38	0/30	0	-	-	-	-	82
	37.5	10/30	33	19.8	16.8	13-23	24	
	150.0	19/30	63	17.7	14.1	10-28	51	
B6C3F1 (Cum x Mai)	9.38	4/21	19	21.8	21.0	21-24	13	12
	37.5	19/30	63	20.5	18.6	8-29	44	
	150.0	25/30	83	15.7	11.2	9-32	75	

CTR-4 Table 2 Comparison of Type C RNA gs antigen expression among the various strains used for subcutaneous and intratracheal MCA tumor induction.

Mouse Strain	Tissue (40%)	gs Antigen P/T* Untreated Control Mice** 1:2	1:4	1:8	MCA-Tumored Mice*** 1:2	1:4	1:8
C57BL/6Cum	Spleen	0/4			0/5		
	Tumor				1/5	1/5	0/5
C57BL/Cum	Spleen	0/5			0/5		
	Tumor				0/5		
C3H/AnfCum	Spleen	2/4	1/4	0/4	4/5	2/5	2/5
	Tumor				4/5	4/5	4/5
C3H/fMai	Spleen	1/6	1/6	0/6	1/5	1/5	0/5
	Tumor				5/5	5/5	5/5
BC3F1/Cum	Spleen	0/5			1/5	1/5	0/5
	Tumor				1/5	1/5	0/5
B6C3F1/Mai	Spleen	2/5	1/5	0/5	3/3	2/3	2/3
	Tumor				3/3	3/3	3/3
B6C3F1/Cum x Mai	Spleen	2/5	1/5	0/5	No data at this time.		
	Tumor				" " " " "		

*P/T number positive at = 3+ complement fixation/Total number specimens tested.

**Untreated mice sacrificed at 4 weeks of age at time 150µg MCA administered to test mice.

***Tumored mice were sacrificed when MCA subcutaneous tumors were 2 cm in size at 10-15 weeks after MCA treatment.

CTR-4

Table 3.

AHH inducibility of strains of Mice used in CTR-3 and CTR-4.

Mouse Strain	AHH Specific Activity Control	MCA-treated	Inducibility
C3H/AnfCum	4.3	18.4	4.25
C3H/fMai	3.6	14.5	3.96
BC3F_1/Cum	3.4	29.7	8.80
C57BL/6Cum	4.5	28.1	6.30
C57BL/Cum	4.5	31.3	7.03
B6C3F1/Cum*	6.1	33.9	5.58
B6C3HF_1/Mai	5.0	41.6	8.32

*C57BL/6Cum and C3H/AnfCum were bred in our laboratory.

CTR-5 August 19, 1974

Relationship Between the Sensitivity to MCA-Induced Squamous Cell Carcinomas and Inducibility of AHH Activity

Objectives:

To determine the role of AHH in carcinogenesis induced by MCA.

Procedure:

C57BL/6 and DBA/2 strains of mice have been mated to obtain F1 and backcross animals. AHH inducibility segregates as a single autosomal gene in this cross. 500µg MCA in 0.02 ml of sterile 0.2% gelatin was given IT to these mice once a week for a total of 3 or 6 weeks.

Progress:

1. The various groups, their date of initiation and relative toxicity are shown in table 1.

2. Animals from selected groups were killed and observed for pathological lesions. The results are in table 2.

3. In the next 30 days all animals will be taken off test, observed macroscopically and processed for pathology.

Conclusions:

These results agree with CTR-3, in that a very low tumor response was initially observed. Discussions with Dr. P. Nettesheim and Mr. W. Blair have indicated that the particle size of the MCA may have been the problem. Our new results (see CTR-3A, B & C) with various doses of MCA using different treatment schedules and vehicles, indicate that conditions can be made whereby viability is high and the number of animals showing early macroscopic lesions are proportionally high. No pathological diagnosis is available at this time. These conditions seem to be 1)large particle size, 2) low pneumonia incidence, 3) careful handling of the individual animals, and, 4) use of older (10-12 week old) animals. This genetic experiment is being repeated in CTR-39.

CTR-5

Table I

Intratracheal Inoculation of Mice. Losses During Treatment Period.

Strain		T#	Treatment	Date Initiated	Initial #Mice	Time on Test	Age on Test	Animals lost during treatment 1x	2x	3x	# Remain on Test 8/20/74
DBA/2	♂	0269	Gel 500 MCA 3x	5/10/73	60	46 wks.	8-9 wks.	10	41	9	0
				7/25/73	60	36 wks	12 wks.	1	0	39	20
DBA/2	♀	0270	Gel 500 MCA 3x	5/10/73	60	46 wks.	8-9 wks.	5	3	46	6
B6D2F$_1$	♂	0271	Gel 500 MCA 3x	5/17/73	60	45 wks.	9-10 wks.	0	0	37	23
B6D2F$_1$	♀	2072	Gel 500 MCA 3x	5/17/73	60	45 wks.	10-11 wks.	0	0	60	0
C57BL/6	♂	2076	Corn Oil 375 MCA 3x	6/13/73	65	41 wks.	8-9 wks.	0	2	41	22
C57BL/6	♀	2077	"	6/13/73	65	41 wks.	8-9 wks.	5	4	32	24
C57BL/6	♂	0286	Corn Oil 3x	6/27/73	10	39 wks.	10 wks.	0	0	5	5
C57BL/6	♀	0287	Corn Oil 3x	6/27/73	10	39 wks.	10 wks.	3	0	4	3
B6D2 D2	♂	0288	Gel 500 MCA 3x	7/26/73	9	36 wks.	7-12 wks.	0	0	8	1
				8/ 2/73	10	35 wks.	7-12 wks.	0	0	9	1
				8/10/73	4	33 wks.	7-12 wks.	0	1	3	0
				11/21/73	19	19 wks.	7-12 wks.	0	0	13	6
B6D2·D2	♀	0289	Gel 500 MCA 3x	7/26/73	8	36 wks.	7-12 wks.	0	0	4	4
				8/ 2/73	2	35 wks.	7-12 wks.	0	0	2	0
				8/10/73	7	33 wks.	7-12 wks.	0	3	4	0
				11/21/73	6	19 wks.	7-12 wks.	0	0	4	2
B6·B6D2	♂	0291	Gel 500 MCA 3x	7/26/73	26	36 wks.	7-12 wks.	0	0	15	11
				8/ 2/73	16	35 wks.	7-12 wks.	0	0	13	3
				8/10/73	3	33 wks.	7-12 wks.	0	0	3	0
				11/13/73	11	20 wks.	7-12 wks.	0	0	8	3
				11/21/73	6	19 wks.	7-12 wks.	0	0	2	4
B6·B6D2	♀	0290	Gel 500 MCA 3x	7/26/73	34	36 wks.	7-12 wks.	0	0	26	8
				8/ 2/73	21	35 wks.	7-12 wks.	0	0	20	1
				11/13/73	11	20 wks.	7-12 wks.	0	0	10	1
				11/21/73	10	19 wks.	7-12 wks.	0	0	7	3
B6D2·B6	♀	0292	Gel 500 MCA 3x	7/26/73	2	36 wks.	7-12 wks.	0	0	2	0
D2·B6D2	♂	0293	Gel 500 MCA 3x	8/ 2/73	8	35 wks.	7-12 wks.	0	0	5	3
				8/10/73	4	33 wks.	7-12 wks.	0	0	4	0
				11/21/73	5	19 wks.	7-12 wks.	0	0	1	4
	♀	0294	Gel 500 MCA 3x	8/ 2/73	5	35 wks.	7-12 wks.	0	0	3	2
				8/10/73	2	33 wks.	7-12 wks.	0	0	1	1
				11/13/73	4	20 wks.	7-12 wks.	0	0	3	1
				11/21/73	2	19 wks.	7-12 wks.	0	0	2	0
D2·D2B6	♂	0315	Gel 500 MCA 3x	11/13/73	6	20 wks.	7-12 wks.	0	0	4	2
D2·D2B6	♀	0316	Gel 500 MCA 3x	11/13/73	5	20	7-12 wks.	0	0	5	0

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CTR-5 Table 2 Pathology Results

Strain	Sex	Time on Test	Rx	# of mice	Results
C57BL/6	♂	182(days)	375 MC IT (3x)	1	Endobronchial abcesses, bronchopneumonia
C57BL/6	♂	287	375 MC IT (3x)	1	Interstitial pneumonia, emphysema
C57BL/6	♂	287	375 MC IT (3x)	1	Interstitial pneumonia, erythroid hyperplasia spleen
C57BL/6	♂	287	375 MC IT (3x)	2	emphysema, pneumonia, erythroid hyperplasia spleen
C57BL/6	♂	287	375 MC IT (3x)	1	normal
C57BL/6	♂	287	375 MC IT (3x)	1	emphysema, adenoma, pneumonia, hyperplasia of spleen
C57BL/6	♂	287	375 MC IT (3x)	1	Interstitial pneumonia, hyperplasia spleen, emphysema
C57BL/6	♂	358	375 MC IT (3x)	1	pneumonitis
C57BL/6	♀	182	375 MC IT (3x)	1	pneumonitis, endobronchial abcesses
C57BL/6	♀	182	375 MC IT (3x)	1	pneumonitis, endobronchial abcesses
C57BL/6	♀	279	375 MC IT (3x)	1	pneumonitis, hyperplasia spleen
C57BL/6	♀	279	375 MC IT (3x)	1	pneumonitis, hyperplasia spleen
C57BL/6	♀	358	375 MC IT (3x)	1	pneumonitis, hyperplasia spleen
$B6D2F_1$	♂	85	500 MC IT (3x)	1	hyperplasia spleen
$B6D2F_1$	♂	85	500 MC IT (3x)	1	lung 80% solid tumor mass
$B6D2F_1$	♂	85	500 MC IT (3x)	7	normal

CTR-5 Table 2: Pathology Results (con't)

Strain	Sex	Time on test	Rx	# of mice	Results
$B6D2F_1$	♂	85	500 MC IT (3x)	1	adenoma, pneumonia
$B6D2F_1$	♂	85	500 MC IT (3x)	1	squamous cell carcinoma
$B6D2F_1$	♂	240	500 MC IT (3x)	1	pneumonia, adenoma, hyperplasia of spleen
$B6D2F_1$	♂	243	500 MC IT (3x)	1	pneumonia, adenoma, lung abcesses, hyperplasia of spleen
$B6D2F_1$	♂	314	500 MC IT (3x)	1	adenoma
$B6D2F_1$	♂	385	500 MC IT (3x)	1	pneumonia, adenomas, lymphocyte neoplasms
$B6D2F_1$	♂	385	500 MC IT (3x)	1	adenocarcinoma, adenoma, red cell neoplasms, type B
$B6D2F_1$	♂	385	500 MC IT (3x)	1	pneumonia, adenoma
$B6D2F_1$	♀	235	500 MC IT (3x)	1	squamous cell carcinoma with marked keratinization - lung infarction
$B6D2F_1$	♀	238	500 MC IT (3x)	1	squamous cell carcinoma
$B6D2F_1$	♀	238	500 MC IT (3x)	1	adenomas, adenocarcinoma with infarction
$B6D2F_1$	♀	380	500 MC IT (3x)	1	pneumonia, adenocarcinoma
$B6D2F_1$	♀	385	500 MC IT (3x)	1	fibrous pneumonia
DBA/2	♂	301	500 MC IT (3x)	1	lymphocyte neoplasm
DBA/2	♂	321	500 MC IT (3x)	1	infarction, BA lesion
DBA/2	♂	321	500 MC IT (3x)	1	normal

CTR-5 Table 2: Pathology Results (con't)

Strain	Sex	Time on test	Rx	# of mice	Results
DBA/2	♀	92	500 MC IT (3x)	4	normal
DBA/2	♀	92	500 MC IT (3x)	2	hyperplasia of spleen
DBA/2	♀	245	500 MC IT (3x)	1	normal
DBA/2	♀	377	500 MC IT (3x)	1	reticulo. cell neoplasm type A; lung, liver lymph nodes w/ giant cells
DBA/2 x D2B6	♂	203	500 MC IT (3x)	1	squamous cell carcinoma
DBA/2 x B6D2	♂	306	500 MC IT (3x)	1	pneumonitis
B6D2 x DBA/2	♀	313	500 MC IT (3x)	1	pneumonitis
B6 x B6D2	♂	41	500 MC IT (3x)	1	lymphoid leukemia
B6 x B6D2	♂	41	500 MC IT (3x)	1	BA lesions, pneumonia
B6 x B6D2	♂	41	500 MC IT (3x)	1	hyperplasia of spleen
B6 x B6D2	♂	41	500 MC IT (3x)	1	pneumonia

CTR-15,16,17 August 20,1974

Effects of TCDD on MCA-Induced Tumor Formation.

Objectives:

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a potent inducer of AHH activity in various mouse strains. A dose of 100nMoles will even induce the MCA-nonresponsive strain, DBA/2. Thus, the DBA/2 strain, possesses the structural genes required for induced AHH activity and lacks (or has a defective) "recognition" protein. A dose of 1nMole TCDD will not induce the DBA/2 strain but will induce the C57BL/6 (our prototype MCA-inducible strain). The susceptiblity of these two strains to MCA carcinogenesis seems to be genetically linked to their ability to be AHH induced with MCA. This study is designed to determine if artificially induced high levels of AHH (induced by TCDD) will alter the susceptibility of either AHH-inducible (B6) or AHH-noninducible (D2) mice.

Progress:

Tables 1 and 2 show the 8 treatment groups for the DBA/2 and C57BL/6 strains, and the weekly tumor incidences for two experiments put on test on separate days. The relative toxicity at 28 days post treatment is also given. The C57BL/6 mice were very sensitive to MCA-induced tumors and no real effect of prior or simultaneous treatment with TCDD was observed (Table 1). The DBA/2 mice were relatively resistent to MCA carcinogenesis and only three treatment schedules yielded tumor incidences of any consequence. Simultaneous treatment with TCDD (especially at 100nMoles) and pretreatment (48 hrs) with the TCDD vehicle, dioxane, enhanced MCA tumorigenesis.

Conclusions:

The results with the TCDD are compatible with the idea that AHH induction (via TCDD) simultaneous with MCA treatment yields more tumors than MCA alone, but the results with dioxane are difficult to assess. Why a 48 hr. pretreatment with 0.010 ml dioxane should enhance MCA-induced tumorigenesis cannot be explained at this time. The fact that both the low and high TCDD levels, when given 48 hrs. before MCA, had no effect, yet dioxane was also in these treatments, indicates that whatever the effect of dioxane, it is cancelled, if TCDD is present. We feel that a repeat of this experiment must be done. The protocol for this repeat is CTR-40.

CTR-15, 16, 17- Effects of TCDD on MCA-induced tumors
(Combined results for mice put on test Oct. 13 & Nov. 9, 1973)

Strain	Treatment -2 days	Treatment 0 day	Toxicity[a] #	Toxicity[a] %	Tu/T[b]	%	Avg. Latency	CI[c]
DBA/2	diox	Trioc	18/40	45	0/22	0	-	-
DBA/2	TCDD(H)	Trioc	35/60	58	0/25	0	-	-
DBA/2	none	150 MCA	15/50	30	1/34	3	217	1
DBA/2	diox	150 MCA	12/40	30	6/25	24	172	14
DBA/2	TCDD(L) +150 MCA	none	11/45	24	5/34	15	199	7
DBA/2	TCDD(H) +150 MCA	none	66/110	60	10/43	23	178	13
DBA/2	TCDD(L)	150 MCA	14/45	31	0/31	0	-	-
DBA/2	TCDD(H)	150 MCA	32/60	53	0/28	0	-	-
C57BL/6	diox	Trioc	1/40	3	0/39	0	-	-
C57BL/6	TCDD(H)	Trioc	33/60	55	0/27	0	-	-
C57BL/6	none	150 MCA	4/40	10	29/36	81	125	65
C57BL/6	diox	150 MCA	7/40	18	24/31	77	119	65
C57BL/6	TCDD(L) +150 MCA	none	18/45	40	27/27	100	132	76
C57BL/6	TCDD(H) +150 MCA	none	37/80	46	33/43	77	123	63
C57BL/6	TCDD(L)	150 MCA	20/45	44	16/23	70	140	50
C57BL/6	TCDD(H)	150 MCA	35/60	58	21/25	84	129	65

[a] Toxicity given in terms of # of mice dead in 28 days.

[b] No. of tumored animals per no. of treated animals 36 weeks after treatment.

[c] Carcinogenic index

CTR-18: Nitrosamine Induced Respiratory Tumors in DBA/2J Mice.

Objectives:

a. To establish toxicity levels for nitrosamines when instilled intratracheally at one or two week intervals several times.

b. To then evaluate the carcinogenicity of these substances in the respiratory tract of DBA/2J mice.

Procedure:

a. The nitrosamines were dissolved in corn oil to give stock solution of 25, 50 and 100 µg/.02 ml of vehicle for each treatment.

b. Groups of mice (50 to 100 each) were intratracheally (I.T.) instilled with three concentrations (25,50 and 100µg) diethylnitrosamine (DEN) at one or two week intervals. Control mice receive 0.02 ml of corn oil alone at each instillation.

Progress:

1. Preliminary trials indicated that 25, 50 and 100µg doses of DEN were not toxic to mice after IT instillation. We therefore initiated our study with chronic weekly doses of 1000 and 2000 µg of DEN per mouse.

2. The early toxic effects after 39 days on test and five treatments were shown in the December report. The higher dosage of DEN (2000µg) was about 5 times more toxic than the 1000µg dosage.

3. Mice received a total of 6 intratracheal (I.T.) injections of DEN and have currently been on test about 10 months (see attached Table 1.). Mice will be held on test and scheduled for gross and histological observation at a later date.

CTR-18
D100

August 19, 1974

Table 1. Survival Incidence Eight Months After Nitrosamine Exposure[a].

Substance Tested	Sex	No. Weeks on Test	Mortality Dead/Tested	(%)	No. surviving Mice
diethyl-nitrosamine 1000µg	♂	43	10/50	20%	40
	♀	"	6/50	12%	44
	Both	"	16/100	16%	84
2000µg	♂	42	6/20	30%	14
	♀	"	11/20	55%	9
	Both	"	17/40	42%	23
Corn Oil .02 ml	♂	42	4/20	20%	16
	♀	"	1/20	5%	19
	Both	"	5/40	12%	35

[a]Mice received 6 intratracheal instillations at weekly intervals.

CTR-18A

August 19, 1974

Nitrosamine Induced Respiratory Tumors in Mice.

Objectives:

To determine the possibility of producing lung tumors with DMN using the wax pellet procedure of Stanton. Dimethylnitrosamine (DMN) and several other nitrosamines are present in small amounts in cigarette smoke. Levels of hydrocarbon hydroxylase activity seem to play a role in the mechanism of DMN carcinogenesis for treatment with polycyclic aromatic hydrocarbon (e.g. MCA) will depress the metabolic activity of DMN by depressing levels of DMN-dimethylase. Therefore, strains most sensitive to MCA (because AHH inducible) may be very resistant to DMN tumorigenesis and vice versa. For this reason both AHH inducible and non-inducible strains have been included.

Procedure:

Since nitrosamines are very volatile the use of IT inoculation procedures as suggested in the initial proposed study was not used since it was felt there was too much danger to the technician. We have substituted the wax pellet technique.

Progress:

Preliminary studies with DBA/2 mice established very quickly that the dose of 1 mg and 0.5 mg was too toxic. We have had some deaths in the control mice due to the nature of the technique. See table 1 for progress in initiating the experiment and the deaths which occurred due to toxicity.

CTR-18A. (C-061)

Aug.14, 1974

Table 1 - DMN Lung Implants in ♀ Mice

Mouse Strain	Treatment	Group	Date on Test	Days on Test	Weeks on Test	# Animals Placed on Test	Death[a]	Tu/T
DBA/2 J	No Carcinogen	01A1A	7/9/74	37	6	30	0	0/30
DBA/2 J	.05ml B:T	01B2A	6/27/74	48	7	30	1	0/29
DBA/2 J	1mg DMN/.05ml B:T	01C4A	6/27/74	48	7	40	40	
DBA/2 J	.5mg DMN/.05ml B:T	01C3A	6/27/74	48	7	40	40	
DBA/2 J	.25mg DMN/.05ml B:T	01C6A	8/2/74	12	2	40	6	0/34
DBA/2 J	.125mg DMN/.05ml B:T	01C5A	8/2/74	12	2	40	4	0/36
SWR/J	No Carcinogen	02A1A	7/23/74	22	4	25	1	0/24
SWR/J	.05ml B:T	02B2A	7/3/74	42	6	30	12	0/18
SWR/J	.25mg DMN/.05ml B:T	02C6A	7/3/74	42	6	40	12	0/28
SWR/J	.125mg DMN/.05ml B:T	02C5A	7/3/74	42	6	40	12	0/28
C57BL/6 Cum	No Carcinogen	03A1A	7/12/74	33	5	30	0	0/30
C57BL/6 Cum	.05ml B:T	03B2A	7/12/74	33	5	30	10	0/20
C57BL/6 Cum	.25mg DMN/.05ml B:T	03C6A	7/16/74	29	5	40	13	0/27
C57BL/6 Cum	.125mg DMN/.05ml B:T	03C5A	7/16/74	29	5	40	7	0/33
BALB/c Mai	No Carcinogen	04A1A	Scheduled					
BALB/c Mai	.05ml B:T	04B2A	for					
BALB/c Mai	.25mg DMN/.05ml B:T	04C6A	Sept. 1974					
BALB/c Mai	.125mg DMN/.05ml B:T	04C5A						

Continued on Page 2.

Table 1 - DMN Lung Implants in ♀ Mice (cont.)

Mouse Strain	Treatment	Group	Date on Test	Days on Test	Weeks on Test	# Animals Placed on Test	Death[a]	Tu/T
C3H/f Mai	No Carcinogen	05A1A	7/12/74	33	5	30	0	0/30
C3H/f Mai	.05ml B:T	05B2A	7/12/74	33	5	30	4	0/26
C3H/f Mai	.25mg DMN	05C5A	7/16/74	29	5	40	11	0/29
C3H/f Mai	.125mg DMN	05C5A	7/16/74	29	5	40	5	0/35

[a]Number of animals dead due to treatment, toxicity or other.

Proposal 1975

A. EXPERIMENT CTR-38 : Effects of Vitamin A on lung carcinogenesis (Substitute for Supplementary study suggested as CTR-32)

1. Purpose: To determine the effects of vitamin A on pulmonary tumorigenesis, AHH inducibility and immunocompetence.

2. Background: Vitamin A is necessary to maintain normal differentiation and function of secretory epithelium. Extensive epithelial hyperplasia associated with anaplasia or squamous metaplasia has been demonstrated in tissue culture by MCA and is similar to that seen in vitamin A deficiency. Both these conditions in tissue culture can be reversed by addition of vitamin A. In hamsters, vitamin A has been shown to inhibit lung tumors by BP as well as forestomach and cervix tumor induction by DMBA or BP. DMBA and BP induced epidemoid tumors in mice and rabbits have been inhibited by retinoic acid.

The mechanism of antitumor effects of vitamin A in animals remains to be defined. There appear to be several areas where vitamin A may play a role in the defense mechanisms of the host to tumorigenesis: (1) The ability of the animal to maintain growth and cellular differentiation. (2) The ability of the microsomal bound enzyme, important in the metabolism of polycyclic aromatic hydrocarbon and nitrosamine carcinogens, to function. Protein- and protein-choline-deficient diets have been shown to influence the enzymatic functions of the cells. (3) The influence of the immune response of an animal by influencing the reticuloendothelial system. Vitamin A has been shown to prevent thymic involvement due to stress and has been important in decreasing the severity of viral infection and tumors of viral origin. (4) The influence of vitamin A on the promoting of mucopolysaccharide biosynthesis or in strengthening of extracellular barriers to chemical and viral involvement.

It is the purpose of these experiments to determine the possibility of increasing susceptibility of mice to lung carcinogenesis by chemical carcinogens. For the present studies we have selected two mouse strains, the C3H/f which is AHH inducible and highly sensitive to subcutaneous carcinogenesis and the DBA/2 which is AHH non-inducible and relatively insusceptible to PAH carcinogenesis. The initial studies will be done with MCA and BP, however, it may prove useful to investigate the significance of vitamin A in nitrosamine and tobacco smoke carcinogenesis based on these initial studies. If we can increase susceptibility to tumor induction by the use of a vitamine A deficient diet we could probably increase our chances of success in the development of mouse inhalation animal model.

3. Materials:

a. Mice
   (1) C3H/f
   (2) DBA/2

b. Chemical Carcinogens
   (1) 0.2% gelatin vehicle
   (2) 250μg MCA/0.02 ml 0.2% gelatin
   (3) 0.6 mg Fe_2O_3
   (4) 1.8 mg BP
   (5) 0.6 mg Fe_2O_3 + 1.8 mg BP

c. Mouse Food
   (1) Vitamin A-free diet
   (2) Vitamin A containing diet

d. Vitamin A trans-retinol (Eastman Kodak Co.)

4. Methods:

a. The most effective way of obtaining a vitamin A deficient animal is to remove the vitamin from the diet of pregnant animals and maintain the mothers and later the offspring on a vitamin free diet. This procedure will be used for evaluation against simply placing 4 week old mice on a vitamin A-free diet at the time of weaning.

b. Mice will be inoculated IT one time every 14 days for 6 to 12 times for MCA and for 10 to 15 times with BP. The number of inoculations will depend on the condition of the animals.

c. The literature indicates AHH induction requires vitamin A. We will include a group of animals maintained on the vitamin A free diet but supplemented with trans-retinol vitamin A 4 hours prior to IT inoculation with the chemical carcinogen.

d. In order to study the effects of vitamin A on AHH induction and to follow the immunological competence in these animals we will sacrifice 3 mice 48 hours after MCA or BP treatment. The lungs and livers will be used for AHH studies while the spleen will be used for cellular immunity studies. Appropriate controls will be included.

e. To study the influence of vitamin A deficiency on the histopathology of the animals, we will sacrifice 3 mice 13-14 days after chemical carcinogen treatment. Appropriate controls will be included. One set of tissues will be kept in the event we wish to pursue scanning electron microscopy at a later date.

f. Vitamin A deficiency will be established by assay of mouse sera or liver tissue.

g. Weight of the animals will be followed to demonstrate effects of avitaminosis.

h. Experimental design:

Group I Mice maintained on Vitamin A diet
- A Vehicle controls
- B Carcinogen treated

Group II Mice maintained on Vitamin A deficient diet
- A Vehicle controls
- B Carcinogenstreated

Group III Mice maintained on vitamine A deficient diet but given trans-retinol Vitamin A 4 hours prior to carcinogen treatment
- A. Vehicle controls
- B Carcinogen treated

Group IV Untreated controls on normal diet

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B. EXPERIMENT CTR-39: Relationship between sensitivity to MCA-induced squamous cell carcinomas and inducibility of AHH.

1. Purpose: Results with CTR-5 yielded no clear-cut answers as to the relationship between genetically mediated levels of AHH and susceptibility to MCA tumors. The tumor response was just too low to make any comparisons. Consultations with Dr. P. Nettesheim and Mr. W. Blair have indicated that the particle size of the MCA may have been the problem. In this study, we intend to use the C3H/f Mai, the DBA/2J and various crosses between these strains to demonstrate the relationship between AHH inducibility and sensitivity to MCA-induced lung tumors.

2. Materials:

a. Mice
(1) C3H/f Mai, 100, ♂, ♀, 8-12 weeks old
(2) DBA/2J ,100, ♂,♀, 8.12 weeks old
(3) C3D2F1, 100, ♂, ♀, 8-12 weeks old
(4) C3D2F1 X D2, 100, ♂, ♀, (all diff. backcrosses)
(5) C3D2F1 X C3, 100, ♂, ♀, (all diff. backcrosses)
(6) C3D2F2, 100, ♂, ♀, (both F2s)

b. Chemicals

-MCA at 250μg/.02ml 2% gelatin

Materials for IT instillation and AHH assay.

3. Methods:

a. At 8-12 weeks of age, give 250μg/.02ml .2% gelatin to mice IT. Mice are to be treated with MCA six times in a twelve week period.

b. At 3 months post-treatment, check every other day for the external symptoms of lung tumors in mice.

c. When external symptoms (severe) are observed, mice will be induced with 80μg MCA/g body weight. and 24 hours later livers will be excised and stored in two pieces in two separate freezers. Gross and histopathological will be done.

d. Need to know precisely the incidence and latency period for each tumor of each group of animals.

e. Must check all mice very closely for external symptoms.

f. At 8 months post-treatment with MCA, take all remaining animals off test: induce with MCA and freeze livers (2 parts)

g. Assay all samples in minimal number of days to provide best analysis of comparative AHH inducibility.

Date on test: 9/2/74

Date off test: about 3/20/75

C. EXPERIMENT CTR-40: Effect of TCDD on MCA carcinogenesis in DBA/2 mice.

1. Purpose: CTR-15, 16 and 17 suggested that TCDD, when given simultaneously with 150μg MCA, would enhance the tumorigenic effects of this dose of MCA. This study is designed to confirm that observation and to determine if MCA and TCDD, given simultaneously in the same vehicle can yield an even higher tumorigenic response.

2. Materials:

a. Mice
(1) 700 - DBA/2 ♀ - 6-8 weeks old.

b. Chemicals
(1) 150μg MCA/.05 ml trioctanoin
(2) 2.4μg TCDD plus 150μg MCA/.01 ml dioxane plus .04 ml trioctanoin
(3) .024μg TCDD plus 150μg MCA per 0.01 ml dioxane plus 0.04 ml trioctanoin
(4) 0.01 ml dioxane plus 0.04 ml trioctanoin

3. Methods:

a. Groups

	Days -2	Days 0		# mice
(1)	none	MCA		30
(2)	Diox	MCA		50
(3)	none	Diox (IP) :MCA (SC)		50
(4)	none	Diox (SC) :MCA (SC)		70
(5)		TCDD (L) :MCA		100
(6)		TCDD (H) :MCA		100
(7)		TCDD (L) :MCA	together	100
(8)		TCDD (H) :MCA	together	100
(9)	TCDD (H)	MCA		100
		Total		700

b. 2 days after TCDD, randomly take two mice per group and freeze the liver for later AHH testing.

c. Making up TCDD:MCA solutions
(1) make up 3.75 mg MCA/ml trioc
(2) make up 240 μg TCDD/ml dioxane

(3) mix 4 parts MCA with 1 part TCDD
∴ 300µg MCA/ml
and 48µg TCDD/ml
-if giving 0.05 ml/mouse
then: 150µg MCA
and 2.4µg TCDD

(4) Control vehicle = 4 parts trioc plus 1 part dioxane

(5) For low TCDD dose, use a 1:100 dilution of the 240µg TCDD/ml solution in dioxane and add 1 part of this dilution to 4 parts MCA (in trioc).

Date on test: 8/21/74
Date off test: 3/21/75

A. EXPERIMENT CTR-41 : Use of inhibitors and inducers of AHH and their effect on MCA induced lung tumors in C3H, B6 and D2 mice.

1. Purpose: Of prime importance is the use of material to epigenetically alter the susceptibility and resistence to chemical carcinogens. Our model system will consist of intratracheal instillation of 250μg MCA every other week for 12 weeks. Inducers and/or inhibitors will be either given previously or simultaneously with the MCA and the role of these chemicals on pulmonary AHH and pulmonary tumors will be evaluated.

2. Materials:

a. Mice
   (1) C3H/f Mai, ♀, 8-10 weeks old
   (2) DBA/2 Cum, ♀, 8-10 weeks old
   (3) C57B1/6 Cum, ♀, 8-10 weeks old

b. Carcinogen: MCA, 250 μg/.02 ml 0.2% gelatin

c. Chemicals:
   (1) TCDD - a potent inducer of AHH
   (2) Phenobarbital - an inducer of constitutive AHH
   (3) 7,8-benzoflavone - an inhibitor of AHH
   (4) 5,6-benzoflavone - an inducer of AHH
   (5) vitamin A - an inducer of AHH
   (6) SKR-525A - an inhibitor of constitutive AHH

3. Methods:

a. The toxic effects of each chemical will be established by determination of LD_{50-20} using 10 mice per chemical (B6 mice).

b. The effects on pulmonary AHH will be established.

c. A maximum of 3 or 4 of the above chemicals will be given 24 hrs prior to MCA treatment and simultaneously with MCA and held for presence of lung tumors.

d. 3-4 months after MCA 10 animals per group will be sacrificed and lungs sent for pathologic and histologic analysis.

e. If the incidence of tumors approximates 50% in the controls then all will be sacrificed.

f. If no tumors, wait till 5 months post-treatment and repeat pathologic tests.

g. Observe # tumors per treated for each group.

B. EXPERIMENT CTR-42 : Determination of the classes of chemical carcinogens and the types of tumors initiated by these carcinogens which are influenced by changes in AHH inducibility.

1. Purpose: In the cross B6D2F1 X D2, AHH inducibility segregates as a single gene yielding 50% of the progeny AHH inducible. This is a perfect population to determine the role of AHH inducibility in cancers induced by classes of carcinogens other than polycyclic aromatic hydrocarbons. Perhaps, in this way, it can be shown that some risk also exists for the non-inducible (or low-inducible) populations.

2. Materials:

a. Mice
B6D2F1 X D2, ♀ and ♂, 4-6 weeks old

b. Chemicals
(1) MCA 250μg/0.02 ml 0.2% gelatin
(2) 2-acetylaminofluorene (AAF)
(3) N-nitrosodimethylamine (DMN)
(4) urethane
(5) N,N-dimethyl-4-aminoazobenzene (DAB)

c. Vehicle
0.2% gelatin in sterile saline

3. Methods:

a. Precheck all mice for AHH inducibility using zoxazolamine-induced sleeping time.
b. Determine LD_{10-20} for each chemical using 10 mice (B6).
c. Give this dose IT to 100 backcross mice.
d. At 6 months take 10 mice off test and do complete autopsy. Check kidney, liver, bladder, as well as lung, for tumors.
e. Take 10 animals per month off test and if 70-80% show signs of tumors take rest of animals off test.
f. Do complete autopsy.

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Schedule

Projected Initiation and Completion Dates of Proposed Experiments

On Going:

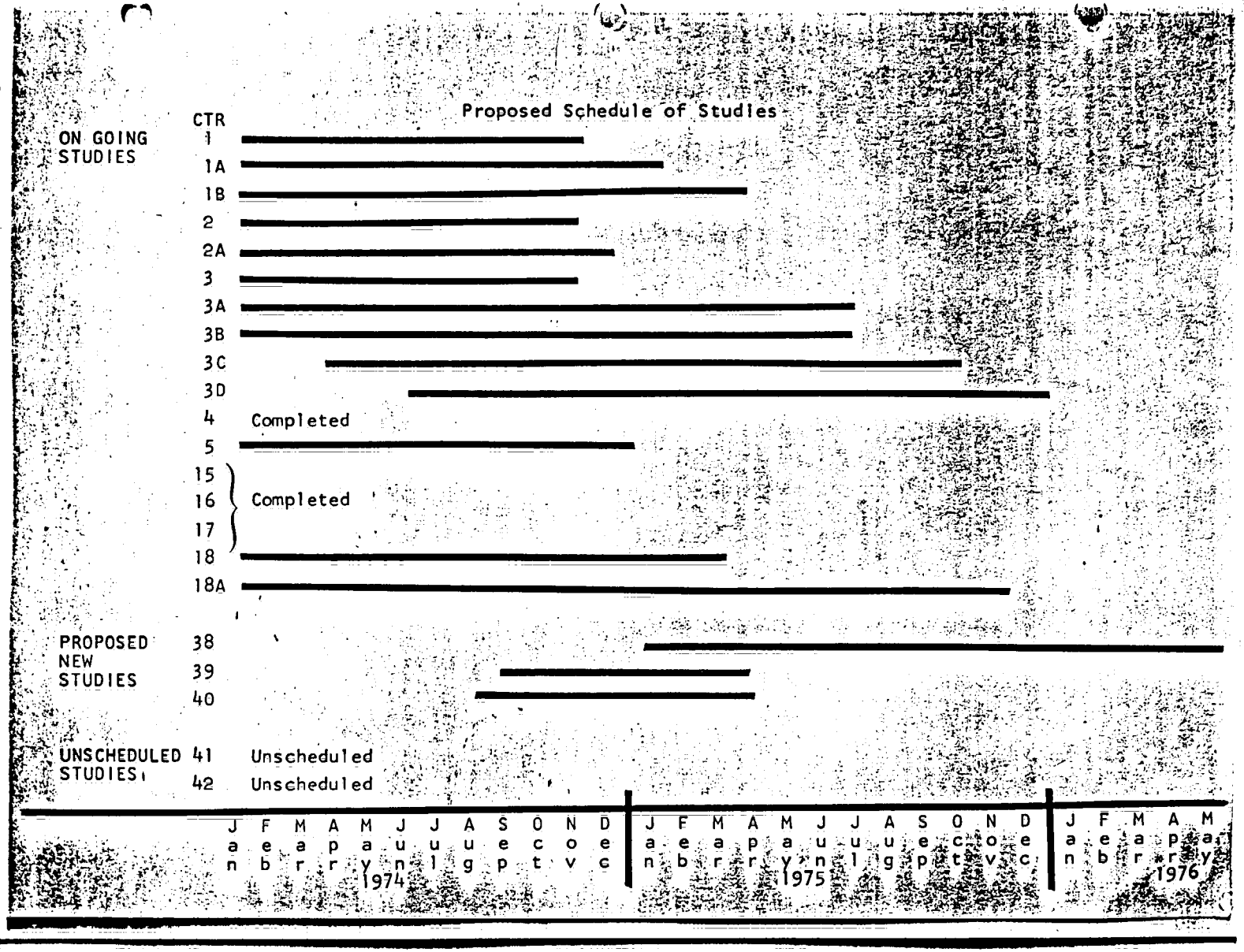
CTR-1:	SC Assay IRI CSC fractions
CTR-1A:	SC Assay IAI CSC fractions
CTR-1B:	SC Assay whole CSC from Dr. Gori
CTR-2:	IP carcinogenesis of nitrosamines
CTR-2A:	Repeat of CTR-2
CTR-3:	IT injection of MCA in C3H/f, C57BL/6, C57BL and hybrid mice
CTR-3A:	Repeat of CTR-3 in C3H/f mice using 2 vehicles, 2 schedules and 2 doses
CTR-3B:	Repeat of CTR-3A in C57BL/6 mice
CTR-3C:	Repeat of CTR-3 in C3H/f ♀ mice using 3 doses for 6-12 weeks
CTR-3D:	Repeat of CTR-3C in C3H/f ♂ mice
CTR-4:	SC-MCA carcinogenesis in C57BL, C3H/f and hybrid mice
CTR-5:	IT injection of MCA in C57BL/6, DBA/2 and hybrid mice
CTR-15:	TCDD effects on MCA carcinogenesis
CTR-16:	
CTR-17:	
CTR-18:	IT DEN injection in DBA/2 mice
CTR-18A:	Wax pellet DMN lung carcinogenesis

Proposed:

CTR-38:	Vitamin A effects on lung carcinogenesis, AHH and immunocompetence
CTR-39:	Repeat of CTR-5 using C3H, DBA and hybrid mice
CTR-40:	Effect of TCDD on MCA Carcinogenesis in DBA/2 Mice

Supplementary Unscheduled Studies:

CTR-41:	Use of Inhibitors and Inducers of AHH and their Effect on MCA
CTR-42:	Other Chemicals in Lung Carcinogenesis.



Budget

B U D G E T

The budget proposed for the contract year 1975 reflects the increases incurred by the massive inflation we are now experiencing and expect to continue during the contract year. This is reflected not only in personnel salaries but other direct costs.

Our cost for plastic cages, animal food and apples as a source of water, have tripled during the past 18 months. We feel this will probably triple again during the next 18 months therefore we are attempting to control this cost by the conversion to permanent cages with automatic watering. The cost of other supplies and mice and the freight on their shipment to us has also increased dramatically.

This budget also reflects the establishing of an in-house computer programing capability and the necessity of renting computer time. We have in the past been able to take advantage of of services available on other contracts.

Due to the long term holding of the mice for chemical carcinogenesis experiments it has been necessary to expand our animal holding facilities and add an additional animal caretaker. The additional facilities will be equipped with shower facilities to meet new government regulations for handling certain chemical carcinogens being used in the CTR Program.

The personnel and positions listed in the budget differ somewhat from that in last year's budget. We have moved personnel and their services within the CTR Contracts in order to establish better budget accountability. The personnel budgets have not however, reflected a dollar change on this basis. The histological service provided in this contract in 1974 Budget has been transferred to another CTR contract instead of splitting it between contracts. This makes for easier accounting procedures. We will provide you with a personnel schedule reflecting the labor distribution between the various CTR-MA contracts.

Renewal for twelve months, Jan. 1, 1975 through Dec 31, 1975

A.	Direct Labor (Schedule A)	$ 52,913.00
B.	Overhead (115% of A)	60,850.00
C.	Other Direct Cost (Schedule B)	23,500.00
D.	Travel	500.00
E.	General and Administrative (16% of $137,763.)	$22,042.00
F.	Total Cost	$ 159.805.00
G.	Fixed Fee	17,755.00
H.	Total Before Equipment	$ 177,560.00
I.	Equipment (Schedule C)	4,000.00
J.	Total Price	$ 181,560.00

Schedule A: Direct Labor

Personnel - Position	Time on Project	Total Hrs.	Rate/ Hr.	Amount $
C. E. Whitmire, Ph.D. Co-Project Director	10%	193	13.51	2,608.00
C. F. Demoise, Ph.D. Assoc. Project Director	50%	482	8.18	7,877.00
M. Haven, M.S. Computer Programer	40%	770	10.58	8,147.00
S. Gosnell, Technician	100%	1926	3.97	7,646.00
Vacancy, Technician	100%	1926	3.97	7,646.00
A. Zuna, Animal Caretaker	100%	1926	3.28	6,317.00
Vacancy, Animal Caretaker	100%	1926	2.75	5,297.00
A. Saborit, Lab. Aide	50%	963	3.17	3,053.00
D. Powers, Adm. Assist.	15%	289	4.21	1,217.00
P. Gradwell, Res. Clerk	50%	963	3.31	3,188.00
B. Ross, Key Punch Oper.	40%	770	3.00	2,310.00
		12,133		$51,372.00
6% Merit Raise (3% for 6 mo.)				1,541.00
Total Direct Labor				$52,913.00

Schedule B: Other Direct Costs

Cages, Mouse Food	$11,000.00
General Supplies	6,000.00
Mice	2,500.00
Computer Time	4,000.00
Total Other Direct Costs	$23,500.00

Schedule C: Equipment

During the next 12 months we will convert from disposable cages to permanent cages with automatic watering to avoid the continued increase in cost of plastic cages and apples as a a water source. The cost of the cages listed above included the cost of plastic cages and the conversion to permanent cages. In addition to this cost we will incur the cost of installing automatic watering to existing racks. Depending on the size of the cage rack the cost varies from $400 - 530/rack. We anticipate converting a minimum of 10 racks to automatic watering for this contract during the 1975 contract year.

$4,000.00

Publications

VII. PUBLICATIONS - 1974

Whitmire, C. E., Demoise, C. F., Kouri, R. E. The Role of the Host in the Development of In Vivo Models for Carcinogenesis Studies. In: Symposium on Experimental Respiratory Carcinogenesis and Bioassays (ed. J.F. PARKS & E. KARBE) Springer-Verlag (in press) 1974

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Kouri, R.E., Demoise, C.F., Whitmire, C.E. The Significance of the Aryl Hydrocarbon Hydroxylase Enzyme Systems in the Selection of Model Systems for Respiratory Carcinogenesis. In: Symposium on Experimental Respiratory Carcinogenesis and Bioassays (ed. J.F. PARKS & E. KARBE) Springer-Verlag (in press) 1974

Kouri, R.E., Ratrie III, H., Whitmire, C.E. Genetic Control of Susceptibility to 3-Methylcholanthrene-Induced Subcutaneous Sarcomas. Int. J. Cancer 13: 714-720, 1974

Kouri, R.E., Kiefer, R., Zimmerman, E.M. Hydrocarbon-Metabolizing Activity of Various Mammalian Cells in Culture. In Vitro (in press) 1974

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Benedict, W.F., Rucker, N., Mark, C., Kouri, R.E. Correlation Between the Balance of Specific Chromosomes and the Expression of Malignancy in Hamster Cells. J. Natl. Cancer Inst. (in press) 1974

Kouri, R.E., Rude, T.H., Thomas, P.E., Whitmire, C.E. Studies on Pulmonary Aryl Hydrocarbon Hydroxylase in Inbred Strains of Mice. (submitted) 1974

Kouri, R.E., Kurtz, S.A., Price, P.J., Benedict, W.F. Studies on the ara-C-Induced Malignant Transformation of Hamster and Rat Cells in Culture. (submitted) 1974

Kouri, R.E. Genetic Control of Susceptibility to Cancer Induced by 3-Methylcholanthrene (MCA) Proceedings of the XI International Cancer Congress, October 1974

VIRUS IN CHEMICAL
CARCINOGENESIS

Material on Jay Levy, M. D., University of California School of Medicine, will be found in Supplement under No. 1011.